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Washed Cotton: Washing Techniques, Processing Characteristics, And Health Effects

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In Cooperation with Cotton Incorporated and the National Institute for Occupational Safety and Health

Editors:
P. J. Wakelyn, R. R. Jacobs, and I. W. Kirk

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Contents

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Preface

Cotton has been a very important textile fiber in the United States for nearly 200 years and is the most widely used textile fiber in the world. However, inhalation of dust in textile manufacturing operations using cotton, flax and soft hemp has been shown to cause an occupational lung disease, byssinosis, in susceptible workers.

There have been federal standards for occupational exposure to cotton dust in the United States since 1971. In 1978, a new standard was promulgated, which lowered the permissible exposure limit and exempted "washed cotton." Earlier studies indicating that washing reduced or eliminated the biological effects of the dust were the basis of this exemption. Thus, washing cotton was considered a promising means for elimination or deactivation of the causative agent(s) of byssinosis if a commercially acceptable method could be developed. As a result, studies of cotton washing were initiated in 1979–81 by an industry/government/union task force with the goal of developing methods that both eliminate the adverse respiratory effects to workers and produce cotton that could be satisfactorily processed in textile mills.

This monograph covers in detail the research on washed cotton conducted by the U.S. Department of Agriculture, the National Institute for Occupational Safety and Health, and Cotton Incorporated with the guidance of the tripartite task force. Washing treatments, processing characteristics, and human health effects studies are discussed.

The editors wish to thank the authors for their dedicated efforts that have produced this monograph.

P. J. Wakelyn
R. R. Jacobs
I. W. Kirk
Editors

Chapter 1
Introduction: Washing Cotton To Remove Potential Health Effects

P. J. Wakelyn[1]

Contents

[1]National Cotton Council, and Chairman, Industry/Government/Union Task for Byssinosis Prevention.

Introduction

In the United States cotton has been a very important fiber and food source and an important source of foreign exchange for nearly 200 years. Cotton continues to be the single most used textile fiber in the world. Its market share of total fiber use is about 50 percent worldwide—53–57 percent in the rest of the world outside of the United States, and 28–30 percent in the United States and Western Europe (*1*). Presently China, the Soviet Union, and the United States account for about 60 percent of the world cotton production of about 82 million bales (39.4 billion pounds; 17.7 billion kg). U.S. cotton production over the last 10 years averaged about 12 million bales (5.8 billion pounds; 2.6 billion kg), grown on about 12 million acres on over 40,000 independent farms in 18 States from California to Virginia. During 1980–84, average annual cotton consumption by U.S. textile mills has been about 6 million bales (2.9 billion pounds; 1.3 billion kg), and the average annual rate of U.S. cotton exports worldwide has been just over 6 million bales. Earnings from U.S. exports of raw cotton during 1980–84 averaged about $2 billion per year, or about 5 percent of the total value of U.S. agricultural exports. In 1984, cotton was fourth ($4.5 billion) among major field crops in value of farm production but first (over $50 billion) at the retail level. There are literally thousands of actual uses—about 100 major uses—for cotton lint in textile items, chiefly in apparel and home furnishings with lesser amounts in industrial fabrics.

Because cotton is such an important agricultural commodity in the United States and the rest of the world, it is important that methods to eliminate health problems associated with conversion of the fiber into yarn be developed.

Why Washed Cotton Studies Were Undertaken

Inhalation of cotton-related dust, in textile manufacturing operations where cotton fiber is converted into yarn and fabric, has been shown to cause an occupational lung disease, byssinosis, in some workers (*2–6*). Byssinosis is characterized in its acute stages by a sensation of chest tightness or shortness of breath, cough, wheezing, and such nonrespiratory complaints as malaise, normally occurring several hours after return to work, after a respite from exposure such as a weekend away from work. Furthermore, acute effects usually can be measured objectively by determining pulmonary function decrements over the work shift. A diagnosis of the symptom complex, byssinosis, cannot depend entirely on the presence of one or more of these respiratory symptoms (*6*). The respiratory symptoms perceived by affected workers vary. They are generally gradual in onset, measured in terms of hours and most marked on the first day of exposure. Depending on which measurements are used (subjective or objective), evidence of "reactor" status appears to be prevalent in some of the general population, although there are undoubtedly marked difference both in the dose necessary to produce the acute response and in the degree of reactivity.

The modern era of concern about the pulmonary effects of inhaled cotton-related dust began with the development of a grading system (table 1.1) for the subjective symptoms of byssinosis by Schilling (*2,7,8*). This was followed by the demonstration of acute effects of inhaled cotton dust on objective measures of airways resistance and ventilatory function (*3*). The first three stages (grades 1/2, 1, and 2) are considered to be completely reversible (*6*), only grade 3 is thought to be a chronic, irreversible state.

The prevalence of byssinosis in cotton textile mills has generally been reported to relate to gravimetric airborne dust concentrations (*4*). So, the major thrust of byssinosis prevention has been engineering control of cotton dust (*9*). However, even at dust concentrations complying with the Occupational Safety and Health Administration (OSHA) permissible exposure limit (see the next section, "U.S. Cotton Dust Standards"), byssinosis can be expected in some workers (*9*), and thus medical surveillance with symptoms questionnaires and spirometry is an additional important occupational health practice in the cotton textile industry.

Table 1.1—*Schilling Clinical Grading System for Byssinosis*

Grade	Symptoms
0	No symptoms
½	Occasional chest tightness or cough on the first day of the working week
1	Chest tightness and/or shortness of breath on every first day of the working week
2	Chest tightness and/or shortness of breath on the first and other days of the working week
3	Grade 2 symptoms accompanied by evidence of permanent loss of lung function

SOURCES: Schilling (*2, 7, 8*).

Furthermore, the adequacy of a gravimetric standard can be questioned on the basis of demonstrated differences in potencies of dusts from various cottons (*6, 9, 10*). Therefore, an alternative approach to control the occurrence of byssinosis, and to keep cotton economically competitive, is to eliminate or deactivate the active agent(s) before the cotton fiber is processed into yarn.

A promising means for elimination or deactivation of the causative agent(s) of byssinosis from cotton is washing. The 1978 Cotton Dust Standard (29 CFR 1910.1043) (*9*) exempted the handling and processing of washed cotton. But OSHA did not define what constituted "washed cotton" other than to say "thoroughly washed in hot water" and known in the trade as "purified or dyed" (*9*). Earlier studies that demonstrated the effectiveness of washing in significantly reducing or eliminating the biological effects of dust were the basis of this exemption (*9*). Workers who process medical-grade cotton (severely washed with hot scour and bleach) were reported to have no symptoms of byssinosis and no acute ventilatory effects (*11*, *12*) despite substantial airborne dust concentrations. A Chinese study, in which cotton was washed prior to manufacturing of blankets (total dust concentration in air of about 48.7 mg/m^3), reported that the dust did not provoke a physiological response (*13*). McDermott et al. (*14*) and Edwards et al. (*15*) reported that, in marked contrast to inhalation of standard cotton dust, experimental inhalation of washed cotton dust did not acutely increase human airways resistance. In addition, Edwards et al. reported that after inhalation of washed cotton dust, the mean 24-hour excretion of the histamine metabolite 1-methyl-4-imidazole acetic acid (Me IAA), in test subjects, was not significantly different from that for unexposed controls (*15*). Merchant et al. found that thoroughly washing cotton prior to processing had two major effects—first, it reduced the amount of dust generated from the cotton fiber during carding; and second, it eliminated the reduction in FEV_1 (forced expiratory volume in 1 second) experienced by a group of human subjects exposed to card room dust who had a significant reduction in FEV_1 when exposed to dust from standard cotton (*16*). Results reported by Haglind and Rylander (*17*) agreed with the findings of Merchant et al.

However, the least severe of the severe washing procedures considered in the 1977 OSHA rulemaking was a batch system using water with a wetting agent and alkali (pH 12) at 100 °C (212 °F) for 30 minutes following steaming. Cotton washed under these severe conditions eliminates the acute respiratory response in exposed workers, but it produces cotton that cannot be processed into yarn on a commercial scale in the textile industry. Thus, severe washing is not a feasible solution to the byssinosis problem in textile mills because of processing difficulties.

After the promulgation of the 1978 Cotton Dust Standard (see next section), there was a renewed interest on the part of the cotton textile industry in the potential for less vigorous washing to remove the active agent(s) from cotton. The cotton industry presented a document entitled "A Critically Needed Research Program To Solve the Cotton Dust/Byssinosis Problem" (*18*) to the Secretary of Agriculture in early 1980, which emphasized washing cotton as a potential solution and outlined a specific plan for accelerating the evaluation of washed cotton. In response, Congress appropriated nearly $1 million a year to the U.S. Department of Agriculture specifically for cotton dust/byssinosis research, which has remained in the USDA appropriations.

Recently, others have pointed to washing as a preprocessing method for cotton to remove the active agent(s). The World Health Organization has stated the following:

> Several studies of cotton washing under controlled exposure have shown that dust and endotoxin levels, respiratory symptoms and decline in lung functions over a shift can all be markedly reduced by washing cotton. It is not yet clear whether this method is technologically acceptable and whether all biological activity can be removed by such a process. There is, therefore, a need for further collaborative studies which should include washing and spinning trials, and acute and prospective evaluation of volunteers exposed to washed cotton (*19*).

Likewise, the National Academy of Sciences, National Research Council Committee on Byssinosis, concluded the following:

> Two emerging technologies that offerpromise of reducing dust concentrations during cotton processing are prior water washing and the application of additives at a low concentration to suppress dusts. The flexibility of these approaches in commercial operations should be explored first in the development and then on the plant scale. The biologic activity of washed cotton and cotton treated with dust suppressants should be determined by the effect of the dust in human volunteers or in a suitable animal model (*6*).

Studies of cotton washing were initiated in 1979 by an industry/government task force. Initial efforts were focused on developing less severe washing methods that would both eliminate adverse respiratory effects and produce cottons that could be satisfactorily processed by textile mills into yarns having acceptable quality. This research, involving experimental exposures to dusts from washed and unwashed cottons, has been conducted at the USDA Agricultural Research Service's, Cotton Quality Research Station in Clemson, South Carolina. For lack of a validated animal model, and to enhance the relevance of the results for the standard setting process, these studies have utilized human volun-

teers to quantify the potencies of various cotton dusts on acute airways effects.

The Industry/Government/Union Task Force for Byssinosis Prevention (as it is now called) is a tripartite committee composed of technical representatives from the cotton and textile industries, USDA, the National Institute for Occupational Safety and Health (NIOSH), and the Amalgamated Clothing and Textile Workers Union (a list of the Task Force members is provided in table 1.2); OSHA representatives attend the meetings as ex officio members. This is the only research effort in the United States in which industry, government, and union are working together to prevent a health problem. The Task Force meets on a regular basis to plan, direct, review, and provide technical and administrative guidance for the research program as it proceeds. This broad-based body was established in 1979 as a government/industry task force at the request of the Secretary of Agriculture to coordinate, refine, and monitor the accelerated cotton dust research efforts and, in particular, the washed cotton research program. The committee was reinstated and formalized as a Government/Industry/Union Task Force in 1981 in accord with a Congressional directive (*20*) associated with the appropriation of funds for research to alleviate the problem of byssinosis. A "Memorandum of Understanding" was developed and signed by the various groups involved, outlining the responsibilities of the participating groups. Cotton Incorporated (CI) has been in charge of the washing efforts, which have included washing on a rayon processing rinse system, a wool scouring system, a batch kier system, and a continuous batt washing system. Medical evaluation of cotton washed by these systems has been carried out by NIOSH with USDA doing the textile processing to generate controlled amounts of dust in the exposure chambers. USDA has evaluated the processing characteristics of cotton washed by the various methods.

Through the efforts of the Task Force, methods of washing cotton have been developed that convincingly demonstrate that washing can reduce acute bronchoconstrictor potency of dust generated during carding of the cotton and have demonstrated that mild washing (essentially water rinsing) can reduce the dustiness of cotton by an average of 50% (range 35–67%). Both effects seem to vary depending on the specific washing methods and the initial potency of the cotton.

However, the washing procedures recommended can adversely affect the processing quality of cotton, so that its potential use by the textile industry may likely be restricted to open-end spinning or to textile mills that produce heavier yarns in which neps and drafting irregularities are less important. The research of the Task Force has provided recommendations (table 1.3) on the definition of "washed cotton" that were well received by OSHA and that OSHA used as a basis for revisions of the cotton dust standard (*21*).

This monograph covers in detail the research on washed cotton conducted by USDA, NIOSH, and Cotton Incorporated under the guidance of the tripartite Task Force.

Table 1.2—Industry/Government/Union Task Force for Byssinosis Prevention

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Table 1.3—Recommendations of Tripartite Task Force for the Definition of Washed Cotton

"I. Since normal scouring, bleaching, mercerizing, and dyeing are more severe than the washing procedures evaluated in the 'Tripartite Studies', cottons processed by these procedures should be considered 'washed cotton' and continue to be exempt from the standard.

"II. OSHA should consider as 'washed cotton', cottons that have been 1) classed as *low middling* light spotted or better, unless spotted, tinged or yellow-stained (described in THE CLASSIFICATION OF COTTON, USDA, AMS, Agriculture Handbook No. 566); and 2) washed on a rayon rinse system or a continuous batt system as used, evaluated, and described (Attachment 2) in our studies and at least 28-50 °C with a wetting agent and at a minimum 40:1 water to fiber ratio. Precaution should be taken to limit bacterial growth and endotoxin accumulation in all baths. If these cottons are being processed, the only requirement under the cotton dust standard should be medical surveillance, every year. The task force also recommends that environmental monitoring be conducted in mills using cotton.

"For cottons classed below *low middling* and all cottons classed as spotted, tinged or yellow-stained, the dust level should be below 500 microgram/m^3 and they should be at a minimum bleached before being considered 'washed cotton' and subject to medical surveillance requirements every year."

NOTE: See chapter 1 section entitled "Fiber Classification and Characterization."

SOURCE: Industry/Government/Union Task Force (*28*).

U.S. Cotton Dust Standards

Cotton dust exposure was first regulated in the United States in 1969 under the amended Walsh-Healy Act (22). Under that law, which applied only to U.S. government contractors, the 1968 American Congress of Government Industrial Hygienists (ACGIH) list of threshold limit values (TLV), which included 1 mg/m^3 for "cotton dust, raw," was adopted. The ACGIH standard for "cotton dust, raw" (which ACGIH defines as "untreated cotton as it exists in the bale. . .") was based on the work of Roach and Schilling (*23*) in Lancashire cotton textile mills—a horizontal elutriator was used to measure dust. This standard (29 CFR 1910.1000, Table Z–1) was subsequently adopted in May 1971 as an established Federal standard, under section 6(a) of the Occupational Safety and Health Act of 1970. For compliance purposes with this standard, OSHA used a personal sampler that measures total dust. This standard no longer applies in textile mills (since March 27, 1984), but it still applies in the nontextile sectors of waste recycling and garnetting (*21*). Also as of December 1985 it is interpreted as a respirable dust limit (i.e., measured with the vertical elutriator cotton dust sampler).

On June 23, 1978, OSHA published two new standards for occupational exposure to cotton dust (*9, 24*). One covered the cotton ginning industry (29 CFR 1910.1046) and the other, the "general industry standard" (29 CFR 1910.1043), covered all other sectors in which cotton is processed or handled—about 35 industries in all. The standard did not apply to the growing and harvesting of cotton, maritime operations, the handling and processing of woven and knitted fabrics, and "the handling or processing of washed cotton." However, OSHA did not define what constituted "washed cotton" other than to say "thoroughly washed in hot water" and known in the trade as "purified or dyed."

The 1978 standard had three different permissable exposure limits (PEL)—200 μg/m^3 for textile manufacturing operations; 500 μg/m^3 for nontextile operations (including knitting) and for waste houses for textile operations; 750 μg/m^3 for slashing and weaving—and no PEL for ginning. The PEL's were to be determined with the vertical elutriator cotton dust sampler, which has a nominal particle size cutoff level of 15μ. The standards were based mainly on research conducted by Merchant et al. in U.S. textile mills around 1970(*4*).

The 1978 standards were immediately challenged in the courts. Subsequently, court or administrative actions invalidated the standard, or stayed its enforcement, for all sectors except textile manufacturing, slashing and weaving, and some downstream manufacturing operations.

OSHA started a reevaluation of the cotton dust standards in 1981 (*25*) and 1982 (*26*). A proposed revised standard was published on June 10, 1983 (*27*). This proposed 1) to maintain the permissible exposure levels and the methods of compliance as they are in the 1978 standard for yarn manufacturing, and slashing and weaving; and 2) to exempt knitting and hosiery, warehousing, classing, conttonseed processing, waste processing, and other nontextile industries from the 1978 standard (29 CFR 1910.1043), because no evidence of significant health risk exists (a finding OSHA must make for any permanent standard).

The final revised standard was promulgated on December 13, 1985 (*21*). It retained in its entirety the 1978 cotton dust standard (29 CFR 1910.1043), which became effective March 27, 1980 (except for engineering controls which became effective March 27, 1984), for yarn manufacturing, slashing and weaving, and waste houses for textile operations; and the old standard (1 mg/m^3, 29 CFR 1910.1000, Table Z–1), which became effective in May 1971, only for the nontextile industries of waste recycling and garnetting. Also for those waste processing sectors the medical surveillance requirements of the 1978 standard were retained. Cottonseed processing, except for the medical surveillance requirements of the 1978 standard was exempted. Knitting and hosiery, classing, and warehousing are exempt from both standards The standard also does not apply to the handling or processing of woven or knitted materials, to the growing, harvesting, or ginning of cotton, to maritime operations, or to the construction industry. Other changes make the standard more cost effective, performance-oriented, and less burdensome on industry, while guaranteeing continuation of protection for workers.

In addition the revised standard redefined washed cotton, using the recommendations of the Industry/Government/Union Task Force for Byssinosis Prevention. (Table 1.3 contains the recommendations of the Task Force.) Medical grade (USP) cotton, cotton that has been scoured, bleached, and dyed, and mercerized cotton are exempt from all provisions of the standard. The handling or processing of cotton classed as "low middling light spotted or better" that has been washed on a continuous batt system or a rayon rinse system, with water at no less than 60 °C; with a water-to-fiber ratio of no less than 40:1; with bacterial contamination in the wash water controlled, are exempt from all provisions except medical surveillance every 2 years. Cotton of grades lower than "low middling light spotted" that has been washed as specified for the better grades of cotton and has also been bleached are exempt from all provisions of the standard except medical surveillance every 2 years and a permissable exposure limit of 500/μg/m^3.

The causative agent(s) of byssinosis is not known nor is the pathogenesis understood (*6, 29, 30*).

"Cotton dust" is defined in the OSHA standard (*9, 21*) as "dust present in the atmosphere during the handling or processing of cotton which may contain a mixture of substances including groundup plant matter, fiber, bacteria, fungi, soil, pesticides, noncotton plant matter, and other contaminants which may have accumulated during the growing, harvesting, and subsequent processing or storage periods. Any dust present during the handling and processing of cotton through the weaving or knitting of fabrics, and dust present in other operations or manufacturing processes using raw or waste cotton fibers or cotton fiber by-products from textile mills are considered cotton dust . . . Lubricating oil mist associated with weaving operations is not considered cotton dust." OSHA also noted that "the relative proportion of these constituents in 'cotton dust' can vary depending on the type of plant, harvesting and storage methods and cleaning operations."

Botanically, cotton dust can contain varying amounts of all parts of the cotton plant and material from other plants (e.g., grasses) as well as fiber and inorganic material (table 1.4) (*6, 29, 31, 32*). Cotton dust is a heterogeneous, complex mixture of chemicals (table 1.5) and microorganisms which varies in composition and biological activity (*6, 29, 32, 33*). A number of compounds have been proposed as contributing to the biological response to cotton dust (*6, 19, 30, 32*). Most of these compounds are thought to originate from either microbiological contaminants or from cotton plant parts entrained with the harvested fiber. Of the possible microbiological contaminants, endotoxins from gram-negative bacteria have received the most attention. Recent research shows that endotoxin levels correlate better than elutriated dust, with acute changes in FEV_1 (*10*). This could lead to a standard in which endotoxin levels as well as dust levels are considered. Another group of microbiological products that have been postulated to contribute to the actute response are the N-formyl methionyl peptides (NFMP) (*34*). These peptides, unique to bacteria, are chemotactic and, therefore, could potentially recruit effector cells to the respiratory mucosa, if present in cotton dust in sufficient amounts. Proteolytic enzymes are a third component of microbiological origin in cotton dust (*35*).

The concept that microbes or their byproducts are the source of the causative agent in cotton dust is an enticing postulate because of the high levels of contamination and myriads of bioactive components that are produced by microbes. However, the uniqueness of byssinosis to the vegetable fiber industry (cotton, flax, and soft hemp) suggests that chemicals in cotton dust may be a causative agent(s) (*5–7, 19, 29*). Over 30 known natural products in cotton plant parts have biological activity (*36*). These compounds are known to act in a variety of ways that are beneficial to the cotton plant (e.g., natural pesticides), but at the same time many of these compounds, when inhaled, may have the potential to cause detrimental biological reactions in animals and man. Byssinosis may involve several natural products of cotton; because of the diverse activity of these components, they need to be fully evaluated. The most significant of these natural products are terpene aldehydes and ketones (e.g., gossypol and lacinilene-C-methyl ether); condensed tannins (flavonols, formed from catechin and gallocatechin); flavonol glycosides (e.g., rutin, isoquercitrin, and quercetin); and amines (e.g., histamine and 5-hydroxytryptamine).

The mechanisms that result in the symptoms of byssinosis have not yet been defined; however, historically the symptoms have been attributed to either an allergic response, a response to a mediator in the dust, such as histamine, or to the induced release of mediators from effector cells such as neutrophils (*6, 9, 19, 30, 32*). Recent research indicates that byssinosis is most likely not an allergic response but that atopic workers may be more susceptible (*37*).

Table 1.4—Cotton Plant Parts: Possible Plant Trash in "Cotton Dust"

Bract	Seedcoat fragments and meat
Leaf	Motes
Vein material	Lint fragments
Petiole	Shale
Capsule	Sticks and stems
Cotyledon	Wood
Burr, pericarp:	Bark
exocarp	Funiculi
mescarp	Grass
endocarp	

SOURCE: Wakelyn et al. (*29*).

Table 1.5—Compounds Found in Cotton Plant Trash

Carbohydrates:
Cellulose [>20%]
Hemicellulose
Pectins
Mono- and di-saccharides
Lignins [>10%]
Tannins:
Condensed (flavanols formed from catechin and gallocatechin) (~10%)
Hydrolyzable
Phenolic compounds:
Terpenes
Flavanols
Flavonols
Coumarins
Porphyrins
Lipids
Proteins and peptides
Glycoproteins and peptides
Amino acids
Aminosugars
Amines (e.g., histamine and 5-hydroxytryptamine [serotonin])
Inorganic salts
Miscellaneous compounds

SOURCES: National Research Council Committee on Byssinosis (*6*); Wakelyn et al. (*29*).

Raw cotton in its marketed form consists of masses of fibers packaged in large bales (approximately 500 pounds; 225 kg). A single pound of cotton may contain 100 million or more individual fibers. (This section is intended to provide brief general information on cotton; for a more in-depth treatment see *ref. 38–47*).

The cotton plant grows naturally as a perennial but for commercial purposes is grown as an annual. It is a warm-weather plant cultivated mostly in North and South America, Asis, Africa, and India. Botanically, there are four principal domesticated species of cotton of commerical importance: *hirsutum, barbadense, arboreum*, and *herbaceum*. Each one of the commercially important species contains many different varieties developed through breeding programs to produce cottons that are faster maturing and have improved insect and disease resistance, yield, strength, etc.

Gossypium hirsutim, developed in the United States from cotton native to Mexico and Central America, includes all of the many commercial varieties of Upland cotton. Upland cottons, in which fiber lengths or staple lengths vary from about ⅞ to 1-½ in. (22 to 38 mm), now provide over 90% of the world's production of raw cotton and 99% of all cotton grown in United States. This is the dominant cotton processed in the United States textile mills.

Gossypium barbadense is of early South American origin and provides the longest staple lengths, ranging from 1–1/16 to 2 in. (27 to 51 mm) with relatively fine diameters compared to the Upland varieties. Commonly known as extralong staple, Egyptian and Pima, it supplies about 8 percent of the current world production of cotton fiber. Pima is a complex cross of Egyptian and American Upland strains and is grown in the southwestern part of the United States (mainly Arizona and the El Paso area—southern Texas and New Mexico) as well as in South America. Pima has many of the characteristics of the better Egyptian cottons.

The other commercial species, *Gossypium arboreum* and *Gossypium herbaceum*, are native of the Old World (India and Eastern Asia). These Asiatic rough cottons are the shortest staple cottons cultivated (ranging from ⅜ to ¾ in., 9.5 to 19 mm) and are coarse compared with American Upland varieties. Both are of minor commercial importance worldwide.

Before moving into trade channels, cotton must be harvested and ginned to remove seeds and trash. The operations of harvesting and ginning the fiber, as well as cultural practices during the growing season, are very important to the quality of the cotton fiber (*40, 45*). Harvesting, one of the final steps in the production of a cotton crop (*38, 40, 45*), is one of the most important steps, as the crop must be harvested before inclement weather can damage quality and reduce yield. Because of economic factors, virtually all of the crop in the United States and much of the world is harvested mechanically (either spindle harvested or stripper harvested). Mechanically harvested cotton tends to contain more trash and other irregularities than does hand-harvested cotton. Hand-picking, however, is still quite prevalent in the less developed countries and in countries where there is an abundant supply of low cost labor.

Seed-cotton as harvested from the plant acquires commercial value only after ginning which is the separation of seed and trash from the lint fibers (*38, 40, 45*). Trash is also removed from the fiber as part of the cleaning process associated with ginning. There are two major ginning processes: saw ginning, which is used for Upland cottons; and roller ginning, which is used for extralong staple cottons and the short Asiatic cottons. After ginning, baled cotton is sampled so that grade and quality can be determined (see "Fiber Classification and Characterization"). The bales are usually stored in nearby warehousees where they may be recompressed to a higher density. Before the bales are sent to the textile mill, they are merchandised and shipped by a cotton merchant. At the textile mill, cotton fiber is spun into yarn and woven or knitted into products for use by other industries and for the consumer. Fabric is dyed and finished, using hot wet treatments, prior to being converted into the final product.

The cotton fiber is a single cell seed hair about 15 to 24 μm in width and 12 to 60 mm long (*40, 41, 48*). The width-to-length ratio varies from 1:1000 up to 1:5000. It appears under the microscope as an irregularly convoluted tube with a central canal (the lumen) throughout its length (*38, 40, 49, 50*). Fiber formation, which involves two fairly distinct stages, begins on the outermost layer of the cotton-seed. The first stage is the formation of the cuticle and the primary wall. During this time, the full diameter and length of the fiber are reached in about the first 25 days after flowering. The second stage in fiber growth, taking about 30–50 days, is secondly wall formation. This begins a few days before the fiber reaches its full length, about the 18th day after flowering. Layers of cellulose molecules are then deposited on the inside of the primary wall, and this process continues until the boll cracks open (45 to 75 days after flowering). These layers constitute the secondary wall.

The essential elements of the morphology of cotton are (1) the outermost layer, known as the cuticle which is a thin film of pectin and wax; (2) the primary wall, less than 0.5 μm thick, which is composed of a netlike fabric of cellulose in which the fibrils are arranged in a crisscross pattern and is impregnated with waxes and pectinaceous and proteinaceous substances; (3) the cellulose secondary wall which constitutes the bulk of the fiber; and (4) the lumen which is usually open in dry cotton and sometimes contains the remains of the cell protoplasm (*40, 41, 48, 50–52*). The secondary wall is differentiated into three discernible zones. The outermost (S_1) is a very thin (not more than 0.5 μm) layer of fibrils oriented helically. The S_2 zone, situated inside S_1, may be up to 5 μm thick and accounts for the bulk of the cellulose content; the fibrils are in the form of helices. In very mature cotton the S_3 layer, which is not always present in cotton fibers, can be detected adjacent to the lining of the lumen. It somewhat resembles the S_1 layer but is much thinner and more fragile.

Chemical Composition of Cotton Fiber

Raw cotton fiber contains on a dry basis about 90–96 percent cellulose, 1–2 percent nitrogen-containing compounds (reported as percent protein) 0.3–1 percent waxes, 0.7–1.2 percent pectins, and small amounts of organic acids, sugars, and ash-producing inorganic salts (*39–44, 48*). Pigment is a very minor impurity of unknown nature. Variations in these values will arise from differences in soil, climate, weather, farming practices, variety of cotton, and other related factors that strongly influence plant growth and fiber development. After purification (removal of naturally occurring fiber impurities) by scouring and bleaching, cotton is at least 99 percent cellulose. All of the impurities are removed almost completely by boiling the fiber in hot dilute sodium hyproxide under pressure (scouring or kier boiling), then washing thoroughly with water.

Cotton wax is located principally in the primary wall of the fiber. The quantity of wax depends on the surface area of the fibers, with the finer cottons tending to have a larger percentage of wax. The wax is a mixture of high-molecular-weight, long-chain, mainly saturated fatty acids and alcohols (with even numbers of carbon atoms, C_{28} to C_{34}), resins, saturated and unsaturated hydrocarbons, sterols, and sterol glucosides (*53, 54*). In terms of its major components cotton wax appears to be 10–15 percent high-molecular-weight esters (no single ester predominates), montanol, 1-triacontanol, B-sitosterol, and a major unidentified component. Wax interferes with wetting of the fiber and penetration of reagents. Wax, however, serves as a lubricant and is essential for efficient spinning of cotton fiber into yarn. If the wax is removed, reduced in quantity, or structurally altered, prior to processing of the cotton, as happens as a result of some washing treatments, some oil spin-finish must be added to the fiber before it can be efficiently processed. The content and texture of the wax are important in predicting the processing potential of the cotton.

Of all the fiber impurities, the nitrogen-containing compounds constiture the largest percentage when expressed as percent protein (1–2 percent) (*39–44, 48*). Most of these compounds are removed by a mild alkali scour. The nitrogen content of scoured fiber is about 0.035 percent N (about 0.22 percent protein). The nitrogenous material occurs principally in the lumen of the fiber, being protoplasmic residue (*55*); a small portion is also extracted from the primary wall (*56*). Cotton fiber and its primary wall both contain proteins/peptides, free amino acids, and most likely nonprotein nitrogen (*56, 57*). The free amino acids that have been detected are glutamic acid, aspartic acid, valine, serine, and threonine (*57*). The removal of nitrogen-containing compounds should not adversely affect textile processing of the cotton fiber into yarn.

The pectic substance (usually designated as pectin) are located mostly in the primary wall of the fiber. Pectin occurs as free pectic acid (linear polymer of 1, 4–D–galacturonic acid) and insoluble calcium, magnesium, and iron pectates. Removal of pectin does not significantly alter the tensile strength of the fiber and has little effect on textile processing and yarn and fabric properties.

The organic acids in the raw fiber, exclusive of pectic acid, are mostly 1-malic (up to 0.5 percent) and citric (up to 0.07 percent) acids. Analyses indicate that other unidentified acids are present also, totaling some 0.3 percent. The removal of organic acids does not significantly affect textile processing.

The inorganic salts (phosphates, carbonates, and oxides) and salts of organic acids present in the raw fiber are reported as percent ash. Analysis of the ash components, expressed as the oxides of the elements present (excluding chlorine which is expressed as such), shows that potassium and calcium are the principal elements present in the fiber, followed by magnesium, sodium, silicon, phosphorus, chlorine, and sulfur. Iron and aluminum are in the lowest concentration, and copper, manganese, boron, and zinc are present in trace amounts. The ash content of cotton is highly variable in composition and quantity, arising from differences in soil and agricultural practices, as well as in field and handling procedures that affect deposition of material on the fiber. The removal of inorganic salts does not affect processing except when the levels of potassium and sodium are low; then there may be a problem with static.

Many varieties of cotton are grown commercially under a wide range of growing conditions, which result in a great many different grades and qualities of cotton that vary widely in their properties and characteristics (*46, 47*). Fiber quality, which is determined by a classification system, is governed to a considerable extent by the variety and quality of the seed planted, the weather, and the farming practices followed during the period in which the fiber is developed, as well as by methods of harvesting and ginning and a host of other interacting variables (*58–60*). Classification is the description of the quality of cotton and furnishes the basis for buying and selling cotton (*38–40, 61, 62*). Fiber classification also provides textile mills with information that relates to the kind of spinning quality that can be expected from the fiber. There are two methods for estimating fiber quality—expert appraisal by a trained classer and instrumentation. The currently accepted method is expert appraisal, but advances are being made in instrument classing (*38*).

Many different classification systems are used worldwide in the various countries in which cotton is grown (*61, 62*). However, all systems basically measure cotton quality using similar parameters, even though the actual classification scheme may be different. The quality parameters are: 1) amount of foreign matter, 2) preparation (effects of ginning), 3) color and luster (discoloration), 4) fiber length and regularity (feel and appearance), 5) fiber fineness, 6) strength, and 7) the variety, growth area, and how ginned (in some countries these are considered the most important parameters). Saw-ginned cotton (Upland cotton) is classed on a different system than is roller-ginned cotton (longer staple fibers and the very short Asiatic cottons).

The Official Cotton Standards of the United States for the Grade of American Upland Cotton (*61*), also called Universal Standards, are used in most countries to buy U.S. cotton and some other cottons.

Cotton classing is basically a "hand and eye" operation, as "feel" and "look" of the fiber are used to distinguish quality levels. Grade, staple length, and micronaire reading indicate to a large extent the spinning utility and hence the market value of each bale. Cotton may be classed in terms of other standards, or by comparison with types; but in the United States if standards are used, they must be the Official Standards.

Grade is determined through appearance by integration of the three factors of grade—color, leaf, and preparation; *staple length* is measured by pulling out a typical portion of fibers from a sample and comparing it with the official staple type; *micronaire* reading is an airflow measurement that indicates a combination of fiber fineness and maturity.

Color: Cotton is normally white but excess weathering in the field and the action of insects and microorganisms can cause the white cotton to lose its brightness and become darker, disclosed (yellow), and spotted. Discoloration can also be caused by accidental or purposeful introduction of substances to the fiber, e.g., oil or lubricants from harvesting equipment, residues from production and harvest-aid chemicals, and gin additives. Any departure from the normal color usually indicates a deterioration in quality.

Leaf: Cotton can become contaminated by leaf and other plant trash, as well as other trash, through exposure in the field and harvesting methods. Leaf and other trash in cotton must be removed as waste in the manufacturing process. Cottons with the smallest amount of foreign matter, other properties being equal, have the highest grade.

Preparation: Preparation describes the relative neppiness of the ginned lint cotton and the degree of smoothness or roughness of the ginning process. Neps are small tangled knots of fiber probably caused by mechanical processing.

Each factor of grade—color, leaf, and preparation—is judged separately by the classer who integrates his assessment of the three into a composite grade, based on his overall impression. The range of grades in the Official Standards is shown in table 1.6.

Table 1.6—Universal Standards for Grade of American Upland Cotton

Grade name code and equivalent symbol[a]							
Plus	White	Light spotted	Spotted	Tinged	Yellow stained	Light gray	Gray
—	[b]GM (11)	GM (12)	GM (13)	—	—	GM (16)	GM (17)
—	[b]SM (21)	SM (22)	[b]SM (23)	SM (24)	SM (25)	SM (26)	SM (27)
M (30)	[b]M (31)	M (32)	[b]M (33)	[b]M (34)	M (35)	M (36)	M (37)
SLM (40)	[b]SLM (41)	SLM (42)	[b]SLM (43)	—	—	SLM (46)	SLM (47)
LM (50)	[b]LM (51)	LM (52)	[b]LM (53)	[b]LM (54)	—	—	—
SGO (60)	[b]SGO (61)	SGO (62)	SGO (63)	—	—	—	—
GO (70)	[b]GO (71)	—	—	—	—	—	—
—	BG (81)	BG (82)	BG (83)	BG (84)	BG (85)	—	BG (87)

[a]Abbreviations: G = good, M = middling, S = strict, BG = below grade, L = low, O = ordinary.
Grade name: GM = good middling, SM = strict middling, M = middling, SLM = strict low middling, LM = low middling, SGO = strict good ordinary, GO = good ordinary, BG = below grade.

Numbers in parentheses are the numerical code for the alphabetical symbols plus color designation. Example: #46 = a strict low middling cotton with light gray color.

White grades in which the color grade exceeds the leaf grade are designated as "plus." Example: Cotton with middling color and strict low middling leaf is designated SLM plus.

[b]Grade designations for which physical standard boxes are maintained. All other standards are descriptive.

SOURCE: USDA Agriculture Handbook No. 566 (*61*).

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Chapter 2
Washing of Cotton

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Introduction

The processes used for the wet treatment of fiber can be divided into two categories: batch and continuous. The batch process usually employs a vessel large enough to process all the material in a lot at one time. The material generally remains in the vessel until all steps of the wet treatment have been completed. In the continuous process, a flow of material passes through separate equipment stages for the various wet-processing steps. Continuous processing can be subdivided into systems that use 1) wool scouring equipment, 2) rayon rinse equipment, and 3) shallow bed (continuous batt) equipment.

Each of these four systems has been used to wash cotton to prevent the adverse health effects associated with byssinosis. The first trials were made on a rayon washing system at the American Enka Company. Tests were performed at the Lowland, Tennessee, plant and at the pilot facility at Enka, North Carolina. In the second series of tests, cotton was washed on the wool processing system. Initial tests were made at the USDA/Agricultural Research Services Western Regional Research Center in Albany, California. Larger scale tests were conducted at the San Angelo Wool Processing Company in San Angelo, Texas. The third series of tests was conducted in the batch kier systems at Circle Mills in Rome, Georgia. The fourth and final series of washing tests was conducted at the Cotton Incorporated washing facility near Greenville, South Carolina. This system uses a continuous shallow bed for washing cotton. A general description of all four of these types of equiment is given in the following sections of this chapter.

Rayon Rinse System for Washing Cotton

The general configuration of the rayon rinse system used to wash cotton is shown in figure 2.1. A wire mesh belt was used to convey the cotton batt through the system. The batt was made up of four layers of picker laps, each of which weighed 138 g per linear meter and was 28 cm wide. The water was applied to the batt by gravity from stainless steel "rain" pans with perforated bottoms placed about 30 cm above the conveyor.

At the wetout station, water with 0.15 wetting agent (Washaid 1173 by SSC Industries, East Point, Georgia) was applied to each lap as it was unrolled onto the conveyor. Forty percent of the total water was applied during wetout. After wetout, the batt passed through nip rolls that squeezed the batt to approximately 1½ kg of water per 1 kg of fiber. All wetting and finishing agent concentrations used for washing on the rayon system were computed based on the weight of the liquor. Thus the concentration of wetting agent on the fiber after wetout was about 0.22.

Twenty percent of the water was applied by using two pans at the wash station. The batt was again squeezed to about 1½ kg of water per 1 kg of fiber. Forty percent of the water was applied at the finish station. The water contained 0.4 to 0.5 percent of a commercial finish compound. Three finish compounds were tested on this system. They were SSC Finish 641 (SSC Industries, East Point, Georgia). Fiberlube AB (Raytex Chemical Corp., Allentown, Pennsylvania and Lubrisan (Reilly-Whiteman Inc., Conshohocken, Pennsylvania). The SSC Finish 641 compound was chosen for use in tests with the human subjects. The batt was squeezed to approximately 1.35 kg of water per 1 kg of fiber before drying, resulting in approximately 0.6 percent (based on fiber weight) of the finish compound left on the fiber before drying. The amount of wet pickup after the final squeeze rolls seemed to depend on the source of the cotton. Texas cotton had slightly lower pickup, Mississippi cotton had slightly higher pickup, and California cotton was intermediate. As indicated in figure 2.1, water used at the wetout and wash stages was released directly to the sewer, but water from the finish stage was recirculated. Finish concentration was maintained by constantly pumping the required amount of finish into the bath. The concentration was chemically checked each hour, and the entire finish bath was drained and refilled every 12 hours.

Depending on the test, from 50 to 65 kg of water at 66 °C were used for each 1 kg of fiber. The fiber was wet on the conveyor for a total of about 4 minutes. The fiber was opened, then dried in a three-stage tunnel (direct hot air) dryer. Temperatures of the three stages were 90 °C, 80 °C, and 50 °C. The moisture content of the fiber ranged from 10 to 20 percent after the first dryer, from 3 to 10 percent after the second dryer, and from 2 to 5 percent after the final dryer. After drying, the fiber was opened with a spiked beater and baled for shipment.

Washing treatments for experiments using the rayon rinse system are given in table 2.1.

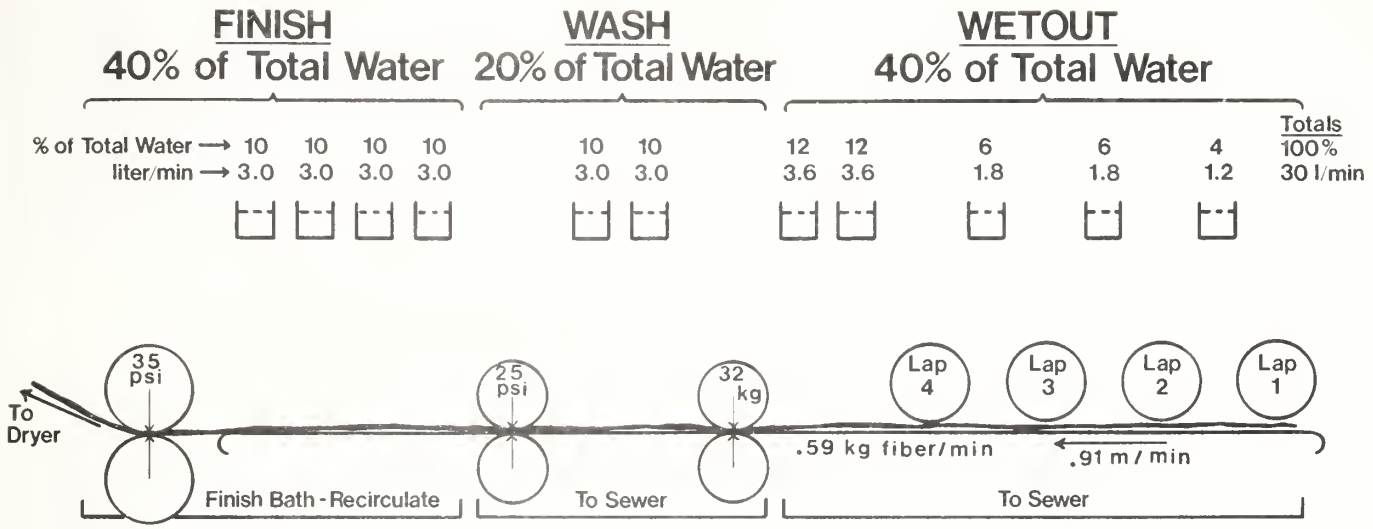

Figure 2.1—Rayon rinse system.

Table 2.1—Washing Treatments Done on Rayon Rinse System

Cotton source	Water-to-fiber ratio	Water temperature	Test ID
Mississippi	50:1	66 °C	MQ–79 G
Texas	50:1	66 °C	MQ–79 E
California	50:1	66 °C	MQ–79 C
Mississippi	65:1	66 °C	MQ–79 J
Mississippi	65:1	28 °C	MQ–79 I

A simplified layout of the full-scale commercial wool scouring system used to wash cotton is shown in figure 2.2. The system contained five "bowls" for wet processing of the fiber. The bowls were 1.22 m (48 inches) wide, the volume of water held by each bowl is shown in figure 2.2. The temperature of the water in each bowl was controlled by steam delivered into the water.

The unwashed cotton was placed in an opening hopper at the head of the washing line. Tuffs of fiber were pulled from the hopper by an inclined spiked conveyor and dropped into the first bowl which contained a 0.2 percent solution (weight of wetting agent to weight of water) of Wash-aid 1173. The cotton was transported down the washing line by raking mechanisms in each bowl (figure 2.3). After each bowl, the fibers passed through nip rolls that squeezed the fiber mass until it contained about 1 kg of water per 1 kg of fiber. In some of the preliminary trials only the last three bowls were used; the cotton was hand fed into the third (or middle) bowl which contained the wetting agent.

In order to maintain the proper water-to-fiber ratios, water was added to the last rinse bowl and pumped counterflow up the washing line to the previous rise bowls. Wetting agent and finish were added to their respective bowls to maintain the proper concentration. The finish used was SCC Finish 641.

Following the squeeze rolls at the final (finish) bowl, the fibers fell into a hopper with a spiked inclined conveyor. This spiked conveyor opened the washed cotton batt and delivered the wet fiber onto the belt of a tunnel dryer. The dryer was heated by steam coils and operated at about 121 °C (250 °F). Because this dryer did not have sufficient capacity to dry the cotton, an additional picker section and two screw conveyor-type trough dryers were added to the system. After it was dried, the washed cotton was baled.

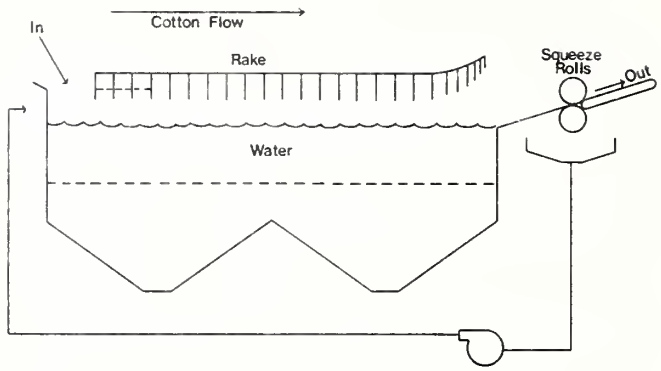

Figure 2.3—Wool scouring bowl.

Because this system required a large volume of water to fill (75,659 gallons; 286,400 l) the water-to-fiber ratios were very high on the first few bales washed. The relationship between the amount of water used per pound of fiber and the amount of fiber that had been washed in the system is illustrated in figure 2.4. In order to obtain the proper water-to-fiber ratio, no water would be added to the system until enough cotton had been washed to reach the desired

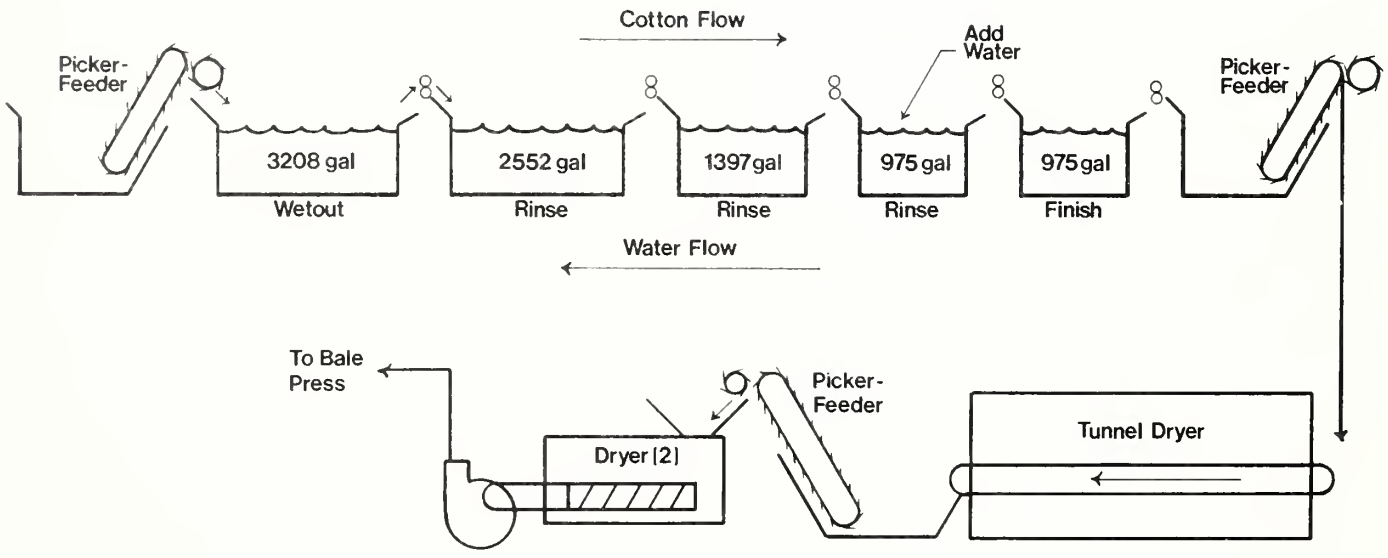

Figure 2.2—Wool scouring system.

water-to-fiber ratio. Then water would be added at a rate to maintain the desired water-to-fiber ratio. For example, if the treatment called for a 40:1 water-to-fiber ratio, water would not be added until after 862 kg (1900 pounds) of cotton had been washed. Then, if cotton was being washed at the rate of 3.6 kg (8 pounds) per minute, 145 kg (320 pounds or 38 gallons) of water per minute would be added to the last rinse bowl.

After preliminary trials, only Mississippi cotton (MQ–80) was washed on the wool scouring system. In all tests, a 0.1 to 0.2 percent solution of Washaid 1173 was used to wetout, and a 0.2 percent solution of Finish 641 was used to finish. All concentrations are based on liquor weight. Washing temperatures and water-to-fiber ratios are given in table 2.2.

Table 2.2—Washing Treatments Done on Wool Scouring System

Water temperature	Water-to-fiber ratio	Test ID
49 °C (120 °F)	40:1	MQ–90 B, D
49 °C (120 °F)	20:1	MQ–90 E, F, H
60 °C (140 °F)	20:1	MQ–91 B
60 °C (140 °F)	40:1	MQ–91 C

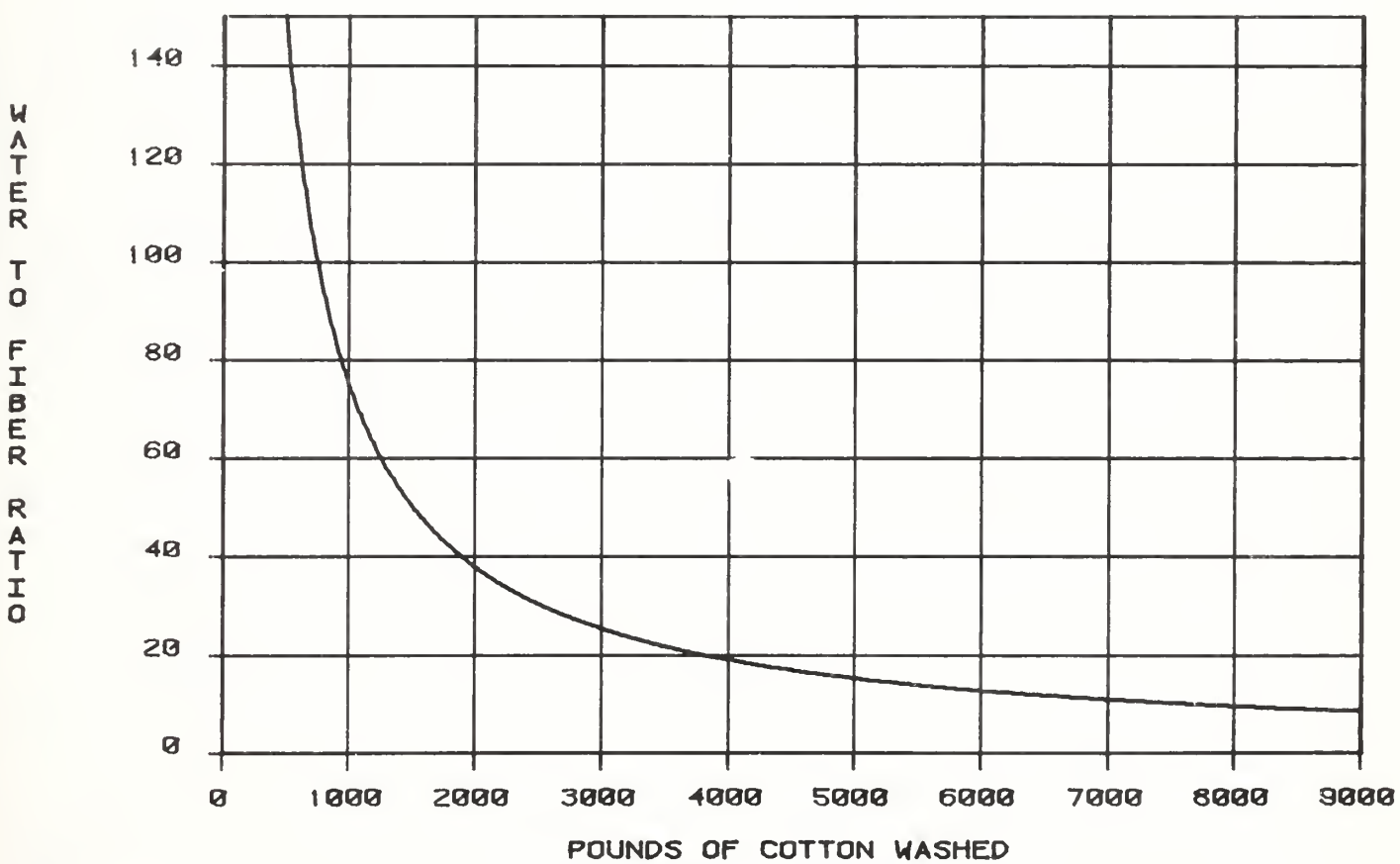

Figure 2.4—Pounds of water per pound of fiber versus the amount of cotton washed in the wool scouring system.

Batch Kier System for Washing Cotton

A simplified diagram of the batch kier system used to wash cotton is shown in figure 2.5. The kier was 2.4 m (8 feet) in diameter and 1.3 m (52 inches) deep. It held approximately 545 kg (1200 pounds) of cotton, which was removed from the bale and packed into the vessel by hand.

Three treatments—wash, scour, bleach—were run in this series of tests. Modifications within these treatments included variations in the water temperature, process time, and amount of water. The general sequence of operations, the compounds used, the time required and temperatures for each treatment are given in table 2.3. A typical time-temperature cycle for the wash treatment is shown in figure 2.6.

After the wash cycle was complete, the "cake" was removed from the kier and placed in a large hopper. The cake of fibers was opened up by rotating spiked cylinders at the end of this hopper. The opened cotton was then delivered onto an apron that fed it through a set of squeeze rolls. After the squeeze rolls, the cotton was picked up by the intake of a fan and delivered into a hopper with a spiked apron that fed the dryer. The dryer was a nine-section tunnel dryer that operated at approximately 143 °C (290 °F). After it was dried, the washed cotton was baled for shipment. Moisture tests on samples as they were removed from the kier showed that the cotton had a wet pickup of approximately 100 percent. After opening and drying, the samples had a moisture content that ranged from 2 to 14 percent. A list of the treatments for the tests conducted on the batch kier system is given in table 2.4.

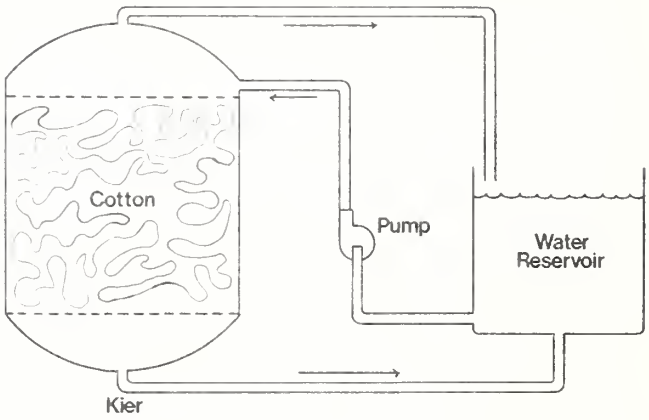

Figure 2.5—Batch kier system.

Table 2.3—Sequence of Operations for Washing on Batch Kier System

Operation	Treatment			Time required
	Wash	Scour	Bleach	
1. Load kier with cotton	X	X	X	30 min
2. Fill kier with water	X	X	X	15 min
3. Add wetting agent	0.5% OWF	0.5% OWF	1.0% OWF	—
4. Bring to temperature	32 °C–60 °C	38 °C	Room temperature.	30 min
5. Add chemicals	None	0.5% sodium carbonate (OWF)	6.5% sodium hypochlorite (OWF)	—
6. Elevate temperature	No	Go to 60 °C	Go to 57 °C	20 min
7. Circulate water solution	10 min	15 min	15 min	(as shown)
8. Rinse (add fresh room-temperature water to top of kier while draining wash solution from bottom)	15 min	15 min	15 min	(as shown)
9. Fill kier with 0.5% solution sodium bisulfide (bleach treatment only)	—	—	Run 10 min	(as shown)
10. Rinse (bleach treatment only)	—	—	Run 10 min	(as shown)
11. Fill kier with water, add SSC Finish 641 for 0.5% solution (OWF), heat to 46 °C, circulate 10 min	X	X	X	20 min
12. Drain finish solution; blow with compressed air; remove washed cotton from kier	X	X	X	30 min

Table 2.4—Tests Run on the Batch Kier System

Treatment and test ID	Wetting agent	Water temperature	Finish
Wash only (MQ–95 C,D,H)	0.1% Washaid 1173	32 °C	0.5% SSC Finish 641
Wash only (MQ–95 E,F,I)	0.1% Washaid 1173	60 °C	0.5% SSC Finish 641
Wash only (MQ–101 I)	1.0% Carawet CM	60 °C	Caralube CM–1
Wash and bleach (MQ–101 J)	1.0% Carawet CM	57 °C	Caralube CM–1
Scour and rinse (MQ–101 K)	1.0% Carawet CM	60 °C	Caralube CM–1

NOTE: The Carawet and Caralube chemicals are from Cardihal Chemical, Inc., Calhoun, Georgia.

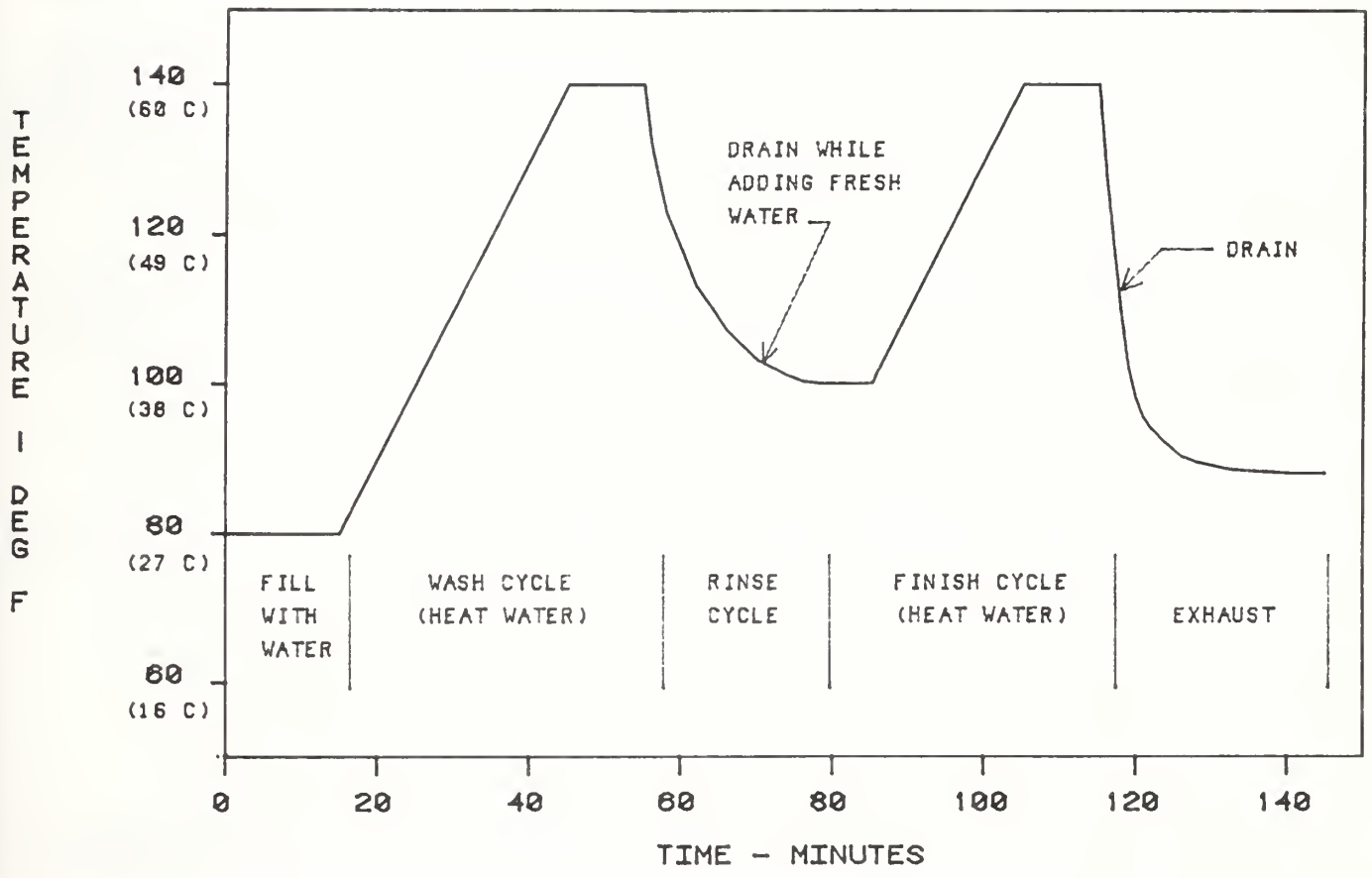

Figure 2.6—Typical time-temperature sequence for batch kier system.

Cotton Incorporated Continuous Batt System for Washing Cotton

The overall process is diagramed in figure 2.7 and has been described by Winch.[3] In general, the process is one in which the fiber is blended, opened, cleaned, formed into a batt, washed, dried, the batt opened, and the fiber rebaled. In the dry mechanical processing of the fiber in preparation for washing, the loose cotton was placed by hand into four hoppers with spiked inclined conveyors. All four hoppers fed simultaneously onto a conveyor belt for blending. The blended fiber then passed through two stages of inclined step cleaners in which the grid bars had been adjusted for minimum cleaning. Because the overall purpose of these tests was to test washing efficiency as opposed to mechanical dry cleaning efficiency, all opening and cleaning equipment was adjusted for minimum cleaning so that the cotton would be opened but the trash was not removed. Next the fibers passed through a fine opener to prepare them for cleaning; then they went into the reserve hopper.

To reduce the amount of mechanical cleaning to the dry fiber, two (of four) rolls were removed from the COTTONMASTER® cleaner (described by Winch). The fiber was fed from the reserve hopper, passed through the two-roll COTTONMASTER®, and the batt formed. The batt weight was controlled at 0.75 kg/m^2. This concluded the dry processing of the fiber.

The wet processing or washing part of the test setup is also diagramed in figure 2.7. Because this facility was designed for continuous processing of a batt, all fiber in all tests reported here passed through all stages of wet processing. The impregnators and rinsers are the shallow-bed devices discussed in detail by Winch. These rinser/impregnator baths were designed to flush out particulate matter that was entrained in the lint. In some tests we attempted to minimize the removal of dust particulate from the lint by removing some of the squeeze rolls from the baths.

Three different washing treatment were used. The solution setup used for each treatment is shown in table 2.5. The concentrations given in the table are based on the weight of fiber processed with 125 percent wet pickup. In all tests a finish was applied to the batt in the finish impregnator. The finish used was a 0.2 percent solution on the weight of the fiber of SSC Finish 641. The dwell times and temperatures for each bath are shown in figure 2.7. Approximately 46 kg of water per 1 kg of fiber were added to the second stage rinser during all washing tests. This water was pumped upstream and used in the first stage rinser before being exhausted from the system.

As the batt left each impregnator or rinser, it passed through nip rolls that squeezed the batt until approximately 1 kg of water per 1 kg of fiber remained in the batt. The batt was plated onto the steam ager conveyer to obtain the dwell

[3]Winch, Allen R., Evaluation of cleaning and washing processes for cotton fiber, Part II: Washing processes and equipment. Text. Res. J., Feb. 1980, pp. 64–72.

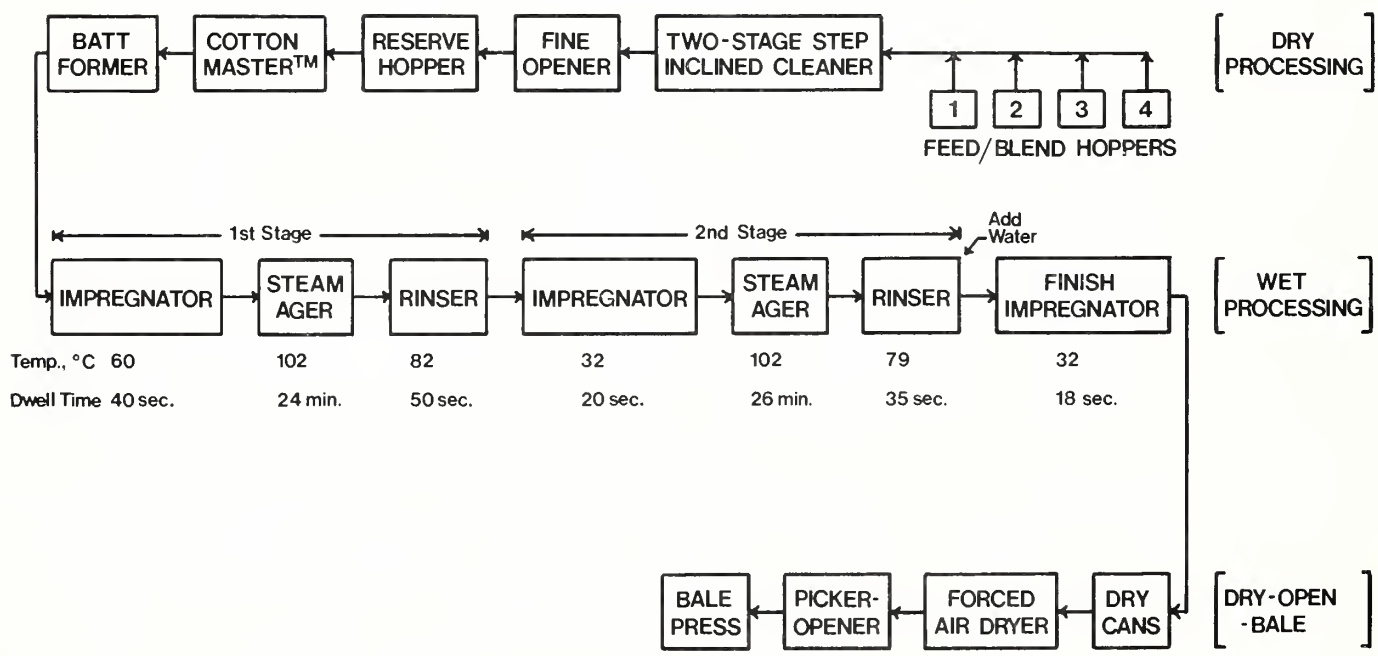

Figure 2.7—Continuous batt process setup for washed cotton tests at Cotton Incorporated, Fiber Processing Center, Greenville, South Carolina.

times given in figure 2.7. The steam agers were operated slightly above atmospheric pressure so that no air would enter at the ports where the batt entered and exited the steamers.

The wash-only treatment is a mild washing treatment for this system. It involves wetting the fiber with the wetting agent at the first stage impregnator and using only water in all other rinsers and impregnators as the batt passes through the system. The only way to achieve a milder washing process in this system would be to eliminate heating the water and forgo use of steam in the agers. The scour-and-bleach treatment is the most severe washing treatment used in these tests. This treatment would produce USP grade scoured and bleached cotton if the fibers had been mechanically drycleaned to the full extent possible by the system. The wash-and-bleach treatment is considered the intermediate level of washing in these tests.

Tests were conducted on two grades of cotton. Both groups of cotton were spindle picked from Mississippi. The better grade (strict low middling) was from the 1980 crop. The lower quality cotton was from the 1982 crop and ranged in grade from strict low middling spotted to low middling spotted. The bales of each group were preblended before they were washed. Table 2.6 gives the combinations of treatments and cottons washed on the continuous batt system.

In order to determine whether the residual activity of the dust in the washed cotton could be further reduced, the lower grade cotton (LMSP) was washed with and without recirculating the finish rinse. Both wash-only and wash-and-bleach treatments were used to wash the fiber. Before each test, the finish impregnator was drained, rinsed, and filled with a solution of water and Clorox brand home laundry bleach (sodium hypochorite). This solution was circulated for 5 minutes. The impregnator was then drained, rinsed, and filled with water which was circulated for 5 minutes. This rinse water was then drained, and the impregnator rinsed and filled with finish solution. A continuous supply of finish rinse was obtained by adding 7.7 kg (17 pounds) of water and 3.1 kg (6.9 pounds) of finish solution per minute to the finish impregnator while washing at the rate of 2.5 kg (5.5 pounds) of fiber per minute. The 10.8 kg (23.9 pounds) per minute of finish solution overflowed from the finish impregnator to the sewer.

Table 2.5—Solution Setup for Each Washing Treatment[a]

Treatment	1st stage impregnator	2nd stage impregnator
Wash only	0.31% Washaid 1173	Water
Wash and bleach	0.31% Washaid 1173	1.2% hydrogen peroxide 0.3% caustic 1.0% silicate
Scour and bleach	7.5% sodium hydroxide 0.6% trisodium phosphate	1.2% hydrogen peroxide 0.3% caustic 1.0% silicate

[a]Concentrations are based on weight of the fiber with 125 percent wet pickup.

Table 2.6—Washing Treatments Done on Continuous Batt System

Cotton[a]	Treatment[b]	Water temperature	Test ID
SLM	Wash only	93 °C	MQ-101B
SLM	Scour and rinse	60 °C	MQ-101 G
SLM	Scour and rinse	93 °C	MQ-101 D
SLM	Wash and bleach	60 °C	MQ-101 H
SLM	Wash and bleach	93 °C	MQ-101 E
SLM	Scour and bleach	93 °C	MQ-101 F
LMSP	Wash only	93 °C	MQ-113 B2
LMSP	Wash and bleach	93 °C	MQ-113 H
LMSP	Scour and bleach	93 °C	MQ-113 I

[a]SLM is Mississippi cotton, 1980 crop, strict low middling grade.
LMSP is Mississippi cotton, 1982 crop, strict low middling spotted to low middling spotted in grade.

[b]See table 2.5 for the setup of these treatments.

Early in our work on washed cotton was realized that cotton fibers that had been washed did not process very well. This was because the washing processes removed the natural lubricants and waxes from the fiber surface. We surveyed companies that market lubricants for textile fibers to determine whether there were commercial finishes available that would allow washed cotton to be processed in the normal or traditional manner. A list of those companies and the trade name(s) for their product(s) is in table 2.7. These finishes were evaluated in a three-step procedure that began with a small-scale test, which used 225 g (approximately 8 ounces) of cotton fiber; continued with a medium-scale test, which used approximately 4.5 kg (10 pounds) of cotton fiber; and ended with a large-scale test, which used approximately 22.5 kg (50 pounds) of cotton fiber. After the finishes were applied to the samples, they were tested either on a full-size production card or on a Shirley miniature card to determine their carding characteristics. Initial testing showed that results from the Shirley card did not correlate well with results from the production card. Therefore, all further tests were run on the full-size card. If a product failed on the small- or medium-scale test, it was generally not tested on a larger scale test. In general the evaluation of a finish on the fiber was a subjective one based on observations of laboratory technicians.

Finish Application Procedures

The samples were prepared for these finish tests by blending Mississippi grown strict low middling cotton (MQ–80) and using the wash-only treatment at 71 °C (160 °F) of the continuous batt system described earlier. No finish was applied. The fiber was dried to 8 to 10 percent moisture content with a maximum dryer temperature of 121 °C (250 °F). The washed fiber was made into picker laps which were subsequently cut into the sample size required for the test being conducted.

In the small-scale test, bath solutions containing 0.5, 1.0, and 1.5 percent of a finish solution based on solids content of the finish were prepared. A 0.1 percent solution of a nonionic wetting agent was added to the bath. The sample was sandwiched between screen wire mesh and immersed into the solution. After wetting the sample was squeezed until it had approximately 100 percent wet pickup from the finish bath. The screen wire would then be removed and the sample dried for 10 minutes at 107 °C (225 °F), after which the samples contained approximately 2 percent moisture. Finally, the samples were labeled and placed in the laboratory for conditioning overnight to equilibrate moisture. As the washing methods were being developed, we realized that in a production system the finishes were more likely to be applied to wet fiber than to dry fiber. Therefore this procedure was modified to prewet the washed fiber and apply the finish wet-on-wet rather than wet-on-dry.

The medium-scale tests using 4.5 kg of fiber were done using basically the same technique to apply the finish. In this case the 45 cm wide picker labs were treated in two 9 m lengths.

The large-scale test usually involved use of 22 kg or more of cotton. These samples were washed using the wash-only treatment and the finish was applied on the continuous batt system (described earlier) at the fiber-processing center at Greenville, South Carolina.

Processing Evaluations

In the general setup, a full-size card equipped with an ASR static meter capable of reading static up to ±10000 volts per centimeter was used. The static meter probe was placed approximately 8 cm above the web. A static eliminator bar that could be switched off and on as desired was installed just above the output side of the crush rolls. Room conditions were maintained at approximately 24 °C (75 °F) and 55 percent relative humidity. Depending on the sample size, the sample was either introduced directly into the card or fed through volumetric hoppers.

The carding production rate was 5.4 kg/h (12 pounds/hour) for the small-scale sample evaluations. For these tests the samples were fed directly into the card without preopening. If the end stayed up, the static voltage was read. After the static voltage was read, the static bar was turned off to see if the sample would still run. Based on visual observation, the technician gave each sample a qualitative performance rating. Before processing of each sample, unwashed cotton was processed through the card to eliminate the possibility of carryover of finish between samples.

The processing evaluations for the medium-scale test were run in a manner very similar to those of the small-scale test. In this case the samples were introduced into the card through the volumetric feed line. The samples were carded at 13.6 kg/h (30 pounds/hour). Again, performance with and without the static bar was noted. The static voltages were recorded, and the quantitative performance rating was made by the operator. In this case, also, unwashed cotton was run through the card before processing of each sample.

For the large-scale evaluation, the procedure was the same as described above for the medium-scale tests except that the waste percentages were noted for the X-L cleaners, flat strips, and under card waste.

If the washed cotton performed well in the large-scale test, yarn was spun from the fiber. Depending on fiber properties, 8/1 Ne, 24/1 Ne, and 40/1 Ne yarn were spun from the samples. This yarn was evaluated for evenness, yarn strength, and grade in our laboratory.

Table 2.7—Textile Lubricant Vendors: Companies That Have Recommended a Finish Candidate for Washed Cotton

Chemical vendor	Product trade name	Qualitative performance rating		
		Satisfactory	Mediocre	Poor
Anscott	Anscolube CO–6		X	
	Anscosoft O		X	
Arkansas Chemical	Luralure			
Armak	RD 5065		X	
	RD 5066		X	
	RD 5067	X		
Arol Chemical	Arolube MIT–1			X
Astro Ind.	Astrolube RF		X	
	Astrolube SP			X
Auralux Chemical	Aurasoft 280		X	
Borne Chemical Co.	Melltone C		X	
Bostik South	S.S. Fiber Oil	X		
Callaway Chemical	Discolube 462		X	
Care Custom Dev.	Fiberlube 8418			
Cavedon Chemical Co.	Cavco JG Wool Oil	X		
Chemical Processing of Ga.	Progalube 39		X	
	Progalube 293		X	
	Progalube 47		X	
	Progalube 266		X	
Chemurgy Products	C-Prolube N			
	C-Prolube 20			X
	C-Prolube N–7			X
	C-Prolube C–51			X
Cindet Chemical	Cinsoft N-BR			X
CNC Chemical	CNC Lube 90	X		
	CNC Napsoft M–100			
Commercial Products Co.	Stockaid A Special			X
Consos, Inc.	Consolube 71	X		
Continental Chemical Co.	Concolube 975			X
American Cyanamid Co.	Cyanatex Softener HP			X
Diamond Shamrock	Napcostat 904			X
	RSF 15		X	
	RSF 17	X		
	RSF 40		X	
	Napcostat CHS			X
Emery Ind.	Emerlube 7486			X
Ethox Chemical Inc.	Cotton Lubricant		X	
High Point Chemical Corp.	Hipochem EM–1			
ICI	Milube C–25		X	
	Milube C–38			
	Milube A–89		X	
	Milube N–30		X	
	Milube N–32		X	
Jordan Chemical Co.	Alubrasol 50–PI		X	
Laurel Products Corp.	Wax Emulsion 103AD			
	Wax Emulsion WG		X	
Lenox Chemical Co.	Lenolube HPE		X	
	Lenolube 100–AS		X	
	Antifly C–5 Conc.			X

Table 2.7—Textile Lubricant Vendors: Companies That Have Recommended a Finish Candidate for Washed Cotton—Continued

Chemical vendor	Product trade name	Qualitative performance rating		
		Satisfactory	Mediocre	Poor
Lutex Chemical Corp.	Magic			X
	Permafin		X	
	Magicstat		X	
Manufacturers Chemical Corp.	Quadrastat S		X	
	Cardlube MWS			
Milliken Chemical	Lubricant PL–710			
	Lubestat 5101		X	
Mona Ind.Inc.	Monamine			
	Monamine CD–100		X	
	Monalube 29–78			X
	Monafax 785			
	Monafax 043		X	
North Chemical Co.	Durolube			X
Organic Chemical Corp.	Orcostat TRI		X	
	Orcostat VS			X
Proctor Chemical Co., Inc.	Antistat 3574			X
	Protolube PE			
Raytex Chemical Corp.	Fiberlube AP	X		
	Fiberlube EPS			X
Reilly Whiteman Inc.	Lubrisan	X		
Richmond Oil Soap & Chem. Co.	Spinlube H–82			X
	Richspin 510L	X		
SSC	Finish 645		X	
	Finish X–246	X		
	Finish 641	X		
Standard Chemical Products	Foryl 100		X	
	Setilon KN			
Star Chemical Co.	Starlube		X	
Superior Ind.	Lube SI 1213		X	
Tanatex	Fibermate CCL			X
Tritex Chemical Corp.	Trisoft NLF		X	
	Trisoft N		X	
Union Carbide	UCON Lubricants 50–HB–260			X
Unitex Chemical Corp.	UL–40			X
Verona (Mobay)	Persoftal FN	X		
	Persoftal FNL			X
York Chemical Inc.	Jaysoft SCO		X	
	Jaysoft NI–M	X		
	Jaylube JK			X
Zimmerman Associates	Zavel			X

Results

Table 2.7 shows the qualitative performance evaluations of the finishes that were tested in the small- and medium-scale tests. About 15 percent of the finishes gave "satisfactory" performance on initial screening. Several of these that were judged best (Cavco JG Wool Oil, Fiberlube AP, Lubrisan, Finish x246. Finish 641, and Jaysoft NI-M) were selected for large-scale tests.

Chapter 3 discusses the processing of washed cotton; therefore, detailed data and analyses will not be given here. However, the general conclusions from these studies are:

1. Card web neps were always higher in washed cotton than in unwashed cotton independent of the finish. However, many of the finishes produced nep readings within an acceptable range.
2. Yarn from washed cotton was generally lower in strength than yarn from the unwashed cotton independent of the finish. These data are quite variable, and the yarn strength is somewhat dependent on the amount of finish applied.
3. The static bar was of considerable help in carding washed cotton at higher rates. However, high static readings do not necessarily indicate that the stock will not process well.
4. The unwashed cotton controls tended to generate negative static readings, whereas the washed samples with finish applied tended to generate positive readings.
5. Finish application and drying are very critical to good processing. Consistent control of each was difficult and probably explains the range of results obtained.
6. "Good" carding results do not necessarily mean that the fiber will give "good" results in other processes.
7. Adding potassium salts, similar to those removed from the fiber during washing, to the finish bath at the rate of 2000 ppm eliminated static problems during carding.
8. In general, no finish at any application level returned the washed cotton to its prewashed condition for efficiency of processing or for yarn quality.

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Chapter 3
Evaluation of Processing Characteristics of Washed Cotton

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Contents

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Introduction

Thoroughly washed cotton was exempted from the Occupational Safety and Health Administration (OSHA) Cotton Dust Standard (*1*). However, washed cotton was not well defined, and it was suggested that only cottons that had been subjected to severe washing conditions qualify for exemption—30 minutes at 100 °C at pH 12 (*2*). Because these conditions are more severe than are economically or technically acceptable to the textile industry, research was conducted to establish less severe washing conditions that would effectively reduce biological activity of cottons to acceptable levels and would leave the fibers in either an enhanced or unchanged state for textile processing. Human exposure studies have shown that washing conditions less severe than those referred to in the OSHA Cotton Dust Standard significantly reduce the biological activity of cotton dust (*3, 4*). Cottons were washed by several methods, potentially useful for commercial application, and the effects on dust levels, fiber properties, and processing quality of the cottons were determined.

Washing Systems and Methods

The details of the various systems and washing conditions are described elsewhere (see chapter 2 and *5–7*). Basically, four washing systems were employed:

1. Continuous rayon rinse system
2. Continuous wool scouring system
3. Batch kier system
4. Cotton Incorporated continuous batt washing system (*7*)

The *rayon rinse system* employs the rain-pan technique of gravity flow wetting of a batt of cotton. On the *wool scouring system,* the cotton is submerged in a relatively large volume of solution and is conveyed by reciprocating rakes and water movement. The *batch kier system* utilizes large stainless steel kiers. The Cotton Incorporated system is a versatile *continuous batt system* in which a batt of cotton passes through a series of bowls and chambers (*7*). The system is designed to effect a scour-and-bleach sequence and is based on traditional pad-steam processes that have been used successfully in the textile industry for many years.

Dust generated in textile processing is reduced significantly by washing (*8–10*). Dust level reductions brought about by moderate washing conditions using only a wetting agent on typical Mississippi cottons are shown in table 3.1. All of the washing systems produced cottons that generated less dust in carding than corresponding unwashed cotton. The average reduction was about 50 percent, and there did not appear to be any major differences in levels of dust reduction that could be related to washing method. The somewhat higher reductions noted for the continuous batt method are partly due to the increased mechanical cleaning inherent in the system.

Dust levels for unwashed and washed cottons for different varieties grown in different locations and that had different initial dust levels are shown in table 3.2. The reductions in dust levels brought about by washing appear similar for the two Mississippi cottons and for the Texas cotton. However, for the California cotton, the dust level reduction brought about by washing was less than that for the other cottons. The initial dust level was also much lower for the California cotton; it is not possible to determine whether the decreased reduction occurred because of the initial low dust level or because of variety or area of growth.

Vigorous opening and cleaning treatments are employed to form the cotton into a batt for washing on the Cotton Incorporated continuous batt system. This mechanical treatment causes a significant reduction in dust level in addition to that brought about by the washing treatment. Table 3.3 shows dust levels for cotton that received minimum cleaning in a typical textile opening line, cotton that received both the minimum cleaning and the additional cleaning (i.e., "Maximum cleaning"), and cotton that received both mechanical treatments and a mild wash.

The maximum cleaning of the stock reduced the dust level by 68 percent, and subsequent washing further reduced the level by 57 percent. The combined effects of maximum mechanical cleaning and washing resulted in an 86 percent reduction in dust level relative to the unwashed cotton that received minimum cleaning.

The effects of diverse washing conditions on dust levels generated by a typical Mississippi cotton washed on the continuous batt system are shown in table 3.4.

The average reduction in dust level was about 55 percent, and there were no practical differences between treatments. This result is generally true for the four washing methods employed. Increasing the washing temperatures and/or adding scouring or bleaching steps have very little effect on dust levels. However, no matter what washing methods or conditions are used, the cotton must be thoroughly wet-out during the process to ensure maximum benefit in dust level reduction.

Table 3.1—Effect of Washing Method on Dust Level Reduction—Mississippi Cottons

Washing method	Dust level reduction (%)
Rayon rinse	48–57
Wool scouring	35–50
Batch kier	45–67
Continuous batt	55–86

Table 3.2—Effects of Area of Growth on Dust Levels by Washing on Rayon Rinse System

Area of growth	Dust level (mg/m^3)		Dust level reduction (%)
	Unwashed[a]	Washed[a]	
Mississippi	1.51 (79–1 A)	0.78(79–1 D)	48
Mississippi	1.38 (MQ–79 F)	0.59 (MQ–79 G)	57
Texas	1.07 (MQ–79 D)	0.59 (MQ–79 E)	45
California	0.58 (MQ–79 B)	0.38 (MQ–79 C)	34

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 3.3—Effects of Vigorous Mechanical Treatment and Washing on Continuous Batt System on Dust Levels

Treatment[a]	Dust level (mg/m^3)	Dust level reduction (%)
Minimum cleaning (MQ–101 A)	1.37	—
Maximum cleaning (MQ–101 L)	0.44	68
Maximum cleaning, plus washing (MQ–101 C)	0.19	86

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 3.4—Effects of Washing Conditions of Continuous Batt System on Dust Levels

Treatment[a]	Dust level (mg/m^3)
Unwashed (maximum mechanical cleaning) (MQ–101 L)	0.44
Wetting agent wash, 60 °C (MQ–101 C)	0.19
Wetting agent wash plus peroxide bleach, 60 °C (MQ–101 H)	0.17
Wetting agent wash plus peroxide bleach, 93 °C (MQ–101 E)	0.23
Sodium hydroxide scour plus peroxide bleach, 93 °C (MQ–101 F)	0.19

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Effects of Washing Methods and Conditions on Fiber Properties

Washing changes the surface characteristics of cotton by removal or reduction of levels of noncellulosic constituents on the fibers. Levels of wax, alcohol extractables, and water soluble reducing substances (WSRS) are shown in table 3.5 for unwashed cottons and corresponding cottons washed using only a wetting agent at 66 °C on the rayon rinse system. If more severe conditions such as high temperature scouring and bleaching are used, the reductions of these substances will be greater (*8, 9*). However, for conditions that are more realistic for washing cotton for use in textile processing—temperature less than 70 °C, use of wetting agent or peroxide bleach only, and minimum agitation—then the levels reported are representative. The wax content was reduced only an average of about 15 percent and alcohol extractables were reduced 47 percent for the Mississippi cotton and more than 60 percent for the California and Texas cottons. Boiling water extractables for another Mississippi cotton similarly washed were reduced 67 percent (not shown in table). WSRS were reduced dramatically to 0.03 percent or less. This reduction in WSRS has been used to aid in differentiation between washed and unwashed cotton (*11*).

One of the major problems encountered in processing of washed cotton is static electricity in the carding and drawing processes. The almost complete removal of water soluble salts from the cotton is probably the major cause of this problem. Thoroughly washed cotton has actually been used as an electrical insulator (*12*). Domelsmith et al. have shown that even very mild washing removes essentially all of the potassium from cotton (*13*). This phenomenon has also been exploited in a method to differentiate between washed and unwashed cotton.

The physical fiber properties of cotton are generally not affected by most washing and bleaching treatments if an effective finish is applied at the proper level. However, any treatments that include a high temperature scour using sodium hydroxide may adversely affect fiber quality even in the presence of a proper finish. Fiber properties are shown in table 3.6 for typical Mississippi cottons washed by the continuous batt method as follows:

1. Unwashed
2. Wash—wetting agent only
3. Wash and bleach—wetting agent plus hydrogen peroxide bleach
4. Scour—wetting agent plus sodium hydroxide
5. Scour and bleach—wetting agent plus sodium hydroxide plus hydrogen peroxide bleach

Table 3.5—Levels of Extractables for Cottons Washed on Rayon Rinse System—66 °C, Wetting Agent Only

Area of growth and treatment[a]	Wax content (%)	Ethyl alcohol extractables (%)	Water soluble reducing substances (%)
Mississippi unwashed (MQ–79 F)	0.46	1.20	0.14
Mississippi washed (MQ–79 G)	0.38	0.64	0.02
California unwashed (MQ–79 B)	0.62	2.72	0.33
California washed (MQ–79 C)	0.51	0.93	0.03
Texas unwashed (MQ–79 D)	0.61	2.26	0.34
Texas washed (MQ–79 E)	0.57	0.88	0.03

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 3.6—Fiber Properties of Washed Cottons

	Fibrograph		
Treatment[a]	2.5% span length (mm)	Uniformity ratio	Strength (g/tex)
Unwashed (MQ–101 A)	27.9	47	24.9
Wash, 93 °C (MQ–101 B)	28.2	47	25.6
Wash, 60 °C (MQ–101 C)	28.2	47	24.2
Wash and bleach, 93 °C (MQ–101 E)	28.2	47	25.0
Wash and bleach, 60 °C (MQ–101 H)	28.4	47	24.3
Scour and rinse, 93 °C (MQ–101 D)	26.9	44	27.3
Scour and rinse, 60 °C (MQ–101 G)	27.7	44	25.8
Scour and bleach, 93 °C (MQ–101 F)	26.7	43	24.5

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Fibers are shorter and less uniform in length for the treatments that include scouring. Even the low temperature treatment that includes scouring appears to adversely affect length uniformity. These results were generally consistent for all washing methods.

Fiber quality is adversely affected by the mechanical treatments associated with two of the washing methods. On the rayon rinse system, the cotton is processed into picker lap for feeding to the wash line; on the continuous batt system, the cotton is fed through a series of opening and cleaning devices and a batt former to prepare the stock for the wash line. An example of the effects that double processing, as required for the rayon rinse system, had on processing and quality of 30s (19.7 mg/m) yarn is shown in table 3.7.

Both yarn appearance factors and end breakage in spinning are adversely affected by the double processing. This problem could possibly be eliminated or minimized by development of systems for feeding these lines that do not require vigorous mechanical action. Improvements in preparation for washing must be forthcoming if these systems are to be used generally to prepare washed cotton for use in textile processing.

Spinning quality of ring-spun cotton yarn is adversely affected by washing, regardless of method. The adverse effect is more pronounced for the treatments that require mechanical preparation of the stock into a lap or batt for feeding to the washing line. The major defects associated with washed cotton are static electricity and altered drafting properties. The static problems are caused by removal of waxes and ionic species from the cotton; drafting problems are caused either by removal or disruption of the natural waxes on the surface of the fibers. Finishes must be applied to the washed cotton to help overcome these difficulties. Finish levels of SSC Finish 641 (SSC Industries, East Point, Georgia) in the range of 0.15–0.50 percent on the weight of the fiber significantly aid the processability of the cotton. Scoured-only and scoured-and-bleached cottons will load severely on the main card cylinder if finish levels near 0.5 percent are not used. For the milder, more practical washing conditions, finish levels below 0.25 percent give the best results. Too much finish will cause lower yarn strength because of increased lubricity of the yarn which promotes fiber slippage when the yarn is stressed.

No finishes evaluated to date completely restore cotton to its unwashed level of processability. Results of processing tests on a typical Mississippi cotton washed at 60 °C by several methods and processed into 30s (19.7 mg/m) yarn are shown in table 3.8.

Table 3.8 shows that card web neps were similar for unwashed cotton and cottons washed on the wool scouring system and by the batch kier method but were much higher for cotton washed by the continuous batt method. For end breakage in spinning, yarn strength, and yarn appearance factors, the unwashed cotton was generally superior to the washed cottons. For the cotton washed by the continuous batt method, the yarn appearance was inferior both to that of the unwashed cotton and to that of the cottons washed by the other methods. This major adverse effect is caused by the mechanical preparation of the stock used in the continuous batt process. As noted earlier, changes in batt preparation are necessary to make cotton washing by this process acceptable for preparing cotton for textile processing.

The effects of high temperature (93 °C) washing, bleaching, and scouring by the continuous batt process on processing quality of 30s (19.7 mg/m) yarn are shown in table 3.9.

These results show clearly the severe adverse effects of scouring treatments on yarn quality. The scoured-only and scoured-and-bleached cottons were extremely difficult to process and produced very poor quality yarns. The washed-only and washed-and-bleached cottons were processed with ease and produced yarns of acceptable commercial quality.

The adverse effects noted for ring-spun yarns are reduced or eliminated when coarse yarns are produced by open-end spinning. In some cases, the very clean washed cottons produced yarns that were superior to the yarns spun from corresponding unwashed cotton. Use of washed cottons in open-end spinning could be of practical value (*14*).

Table 3.7—Effects of Double Processing on Carding and Spinning Performance of Cotton

Treatment[a]	Card web neps per 645 cm^2	Spinning EDMSH[b]	Yarn break factor (units)	Yarn neps per 914 m
Unwashed (79–1A)[2]	35	41	1799	1960
Unwashed (79–1A), Double processed	78	66	1789	2534

[a]Designated in parentheses refer to cotton ID (see app. 2).
[b]Ends down per 1000 spindle hours.

Table 3.8—Processing Qualities of Cottons Washed by Different Methods

Washing method and treatment[a]	Card web neps per 645 cm^2	Spinning EDMSH[b]	Yarn break factor (units)	Yarn neps per 914 m	Yarn appearance	
					Index	Grade
Unwashed (MQ–91 A & MQ–101 A)	15	19	1973	881	103	C^+
Wool scouring (MQ–91 B, C)	16	50	1812	1145	88	C
Batch kier (MQ–91 F)	12	64	1793	1029	103	C^+
Continuous batt (MQ–101 C)	80	19	1877	2364	60	BG[c]

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Ends down per 1000 spindle hours.
[c]Below grade.

Table 3.9—Effects of Washing Conditions on Processing Quality

Treatment[a]	Card web neps per 645 cm^2	Spinning EDMSH[b]	Yarn break factor (units)	Yarn neps per 914 m	Yarn C. V. (%)
Wash (MQ–101 B)	67	43	1802	2247	24.9
Wash and bleach (MQ–101 E)	76	17	2162	2330	24.9
Scour (MQ–101 D)	98	70	1633	3514	28.3
Scour and bleach (MQ–111 B)	96	293	1679	5478	30.0

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Ends down per 1000 spindle hours.

Suggestions for Improving Processing Quality of Washed Cotton

Cottons washed by high temperature scouring procedures using sodium hydroxide have altered length and surface characteristics and may be confined in usage to a limited range of yarns and end products. Cottons washed with wetting agent or wetting agent plus peroxide bleach can be made into a wide range of yarns for general textile usage. However, even for these cottons, the processing and yarn qualities are generally somewhat poorer than those of corresponding unwashed cotton.

The three factors that are most often associated with the more serious processing difficulties are static electricity, altered drafting characteristics, and neppiness caused both by mechanical preprocessing and by agitation during washing. The static problems are alleviated to an extent by application of finishes in the final rinse bath of the washing process and use of a static eliminator bar under the fiber web at the card crush rolls. However, additional improvements in the static problem should be possible through formulation of finishes that would restore the ionic characteristics of the fiber surface and provide the proper lubricity for good fiber to fiber cohesion during drafting of the stock.

The mechanical preparation of the cotton stock for feeding the continuous wash lines could be improved to specifically prepare the cotton for subsequent textile processing. The vigorous cleaning treatments and double processing of stock as now employed in these processes to prepare stock for special end usage, such as nonwoven fabrics, would not be required for cottons that will be used in traditional textile yarn manufacturing processes.

Fine tuning of the yarn manufacturing machinery to the peculiar characteristics of washed cotton should lead to improved processability and yarn quality. Combing the washed cotton could remove short fibers and neps that are associated with poor yarn appearance. Blending with polyester or other synthetic fibers may improve processability and produce yarns with desirable properties.

Yarns spun on open-end systems generally have better appearance characteristics than corresponding yarns spun on ring frames. One reason for this is that the forces involved in the yarn assembly process in open-end spinning tend to force trash and neps into the core of the yarn, thus minimizing surface irregularities. Trials using washed cotton in open-end spinning have been successful and could provide a useful outlet for washed cotton.

Summary

Washing cotton by use of both batch and continuous processes and treatments varying from a mild wash with a wetting agent to a severe scour and bleach with sodium hydroxide and hydrogen peroxide reduces dust levels in processing by about 50 percent. Washing cotton tends to lower processing and yarn qualities. The adverse effects are most pronounced with the continuous systems that use a mechanically prepared batt for feeding the wash line and for treatments that include scouring with sodium hydroxide. The washing treatments that use only a wetting agent or wetting agent and hydrogen peroxide bleach are effective treatments that cause minimal adverse effects on fiber properties and processing and on yarn qualities. With some modifications in washing systems and optimization of finishes and the yarn manufacturing processes, it is reasonable to expect that, with the possible exception of very fine yarns, acceptable commercial yarns can be produced from these cottons.

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Chapter 4
Evaluation of Acute Human Airway Toxicity of Standard and Washed Cotton Dusts

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Contents

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Introduction

The major method of byssinosis prevention has been the control of dust concentrations to which workers are exposed (*1*). However, even at dust concentrations complying with the Occupational Safety and Health Administration (OSHA) permissible exposure limit, byssinosis can be expected (*2*), and the adequacy of a gravimetric dust standard can be questioned on the basis of demonstrated differences in potencies of dusts from various cottons (*3*). Therefore, it is important to evaluate another approach to control the occurrence of byssinosis (*4*).

When OSHA promulgated the cotton dust standard, "thoroughly washed" cotton was exempted from coverage because OSHA recognized "the effectiveness of the washing process in significantly reducing or eliminating the biological effects of cotton dust" (*1*). With this, there was a renewed interest in the potential for less vigorous washing to remove the active agent(s) from cotton. So, in 1979-80, an industry/government research effort was started to evaluate washed cotton as a potential solution (*4*).

This research includes studies, conducted at the USDA Agricultural Research Service's Cotton Quality Research Station at Clemson, South Carolina, that utilized human volunteers to quantify the potency of various cotton dusts. This chapter presents methods of dust generation and control, selection of human subjects, and measurement of the airway responses to inhaled cotton dust. It offers a general review of the human ventilatory response results from the experimental human exposure studies involving washed cotton.

Methods

Dust Generation and Exposure Facilities

Five important environmental aspects of the study methods will be addressed: 1) source of clean, conditioned air; 2) dust generation; 3) facilities for exposing subjects; 4) measuring dust concentration; and 5) control of dust concentration. Details have been described elsewhere (*5–7*).

Source of Clean, Conditioned Air.—Supply air to an experimental card room was initially obtained from an adjoining part of the Cotton Quality Research Station facility. Since late 1981, it has been obtained from outside the facility. In both cases, the supply air passed through a high efficiency composition filter and an electrostatic precipitator to remove particulate. Air temperature was controlled to approximately 24 °C (75 °F) by a chilled water coil and a modulated steam heating coil, and relative humidity was controlled to approximately 55 percent by a modulated steam humidifying system within the air inlet ducting system. Sensors for both temperature and humidity controls were located in the experimental card room. The clean and conditioned air was discharged into the experimental cardroom near the ceiling, through vents designed to minimize the effect of air currents on the dust-generating characteristics of the carding machine. Although the flow of supply air could be adjusted, standard conditions called for 18.4 m^3 min during exposures.

Dust Generation.—To generate airborne dust for the experimental exposures, test cottons were carded on a commercial, lap-fed carding machine located centrally in the experimental card room. Carding on dust exposure days started before subjects began their exposures so that the dust concentration would be at the desired level at the beginning of exposures. Carding continued until subjects had completed their exposure.

Facilities for Exposing Subjects.—Until mid-1981, these studies involved exposing the human subjects in the card room itself, which measures 6.25 m by 4.28 m by 3.05 m with a volume of 81.6 m^3. The subjects sat in chairs around the periphery of the room and, because there was measurable nonuniformity of dust concentration in the card room, they were moved from chair to chair at regular intervals so that they made a complete circuit during the exposure period. There were several disadvantages and potential risks involved in exposing human volunteers in this manner. The card room was not designed for human exposure studies and was noisy, crowded, and (even with conventional guards) potentially hazardous in terms of moving machinery parts. Furthermore, the physical layout of the pilot yarn production facility was such that picking, combing, drawing, and roving operations had to be restricted during the human exposure studies to avoid inhalation of dust that was not part of the study. In addition, the subjects could not be "blinded" to whether or not a particular day was a dust exposure day or a "clean room" exposure day. There was obvious visual perception by the subjects of cotton being

processed on the card and of lint ("fly") in the air during a dust day.

In late 1981, a remote exposure facility was constructed adjacent to the pilot mill, and ducts were installed to convey dust from the card room to an exposure room (app. 5). Advantages were obvious: less noise, more room, natural lighting, and no moving machinery. Also, because subjects no longer had to walk through the pilot mill, and because the ventilation system was entirely independent of the rest of the plant, there were no longer restrictions on picking, combing, drawing, and roving activities. Finally, because the duct system acted as a horizontal elutriator, there was little to no "fly" visible in the remote exposure rooms and subjects could be largely blinded to whether or not cotton was being carded on a particular day.

Despite this horizontal elutriator effect, there were only minimal between-room differences in the particle size distribution of elutriated dust. A series of exposure measuring human subjects' FEV_1 (forced expiratory volume in 1 second) responses to "clean room" conditions and to an unwashed cotton dust in the card room and in the remote exposure room showed that both rooms yielded equivalent dose response slopes (-8.7 percent per mg/m^3 in the card room and -7.8 percent per mg/m^3 in the remote room). As an additional precaution to prevent unwarranted differences in the particle size distributions in the remote exposure room, airflow rate through the ducts and the card production rate were kept constant for all tests, regardless of the cotton being processed on the carding machine.

Measurement of Dust Concentration.—During the initial exposure series, when subjects were placed in the card room for exposures, four dust sampling stations were located in the card room. Each consisted of a vertical elutriator suspended from the ceiling and was located in a separate quadrant of the room with elutriator barrel entrance located 2 m above floor level. At this height, the samplers did not obstruct movement of personnel and equipment around the room, and it had been demonstrated that there was no difference between dust concentration at this level and that between 1.37 and 1.68 m above the floor, as required by the OSHA cotton dust standard (*1*). A high volume pump located outside the room was connected to a line serving each elutriator via a short length of plastic tube with a critical flow orifice. Cassettes containing 37-mm diameter, 5-μm pore size, polyvinyl chloride filters were placed at the elutriator exits to capture dust. Approximately every hour during each dust exposure period, new filter cassettes were exchanged for the previously placed cassettes, and the filters were weighed gravimetrically using standard technique in a temperature- and humidity-controlled environment. A mean time weighted average dust concentration for each complete exposure period was determined using weights from all filters from all elutriators, or about 24 filters. During later series of exposures, when the subjects were exposed in a remote room, the dust concentration measurement was accomplished in like manner, but with elutriators placed around the remote exposure room, one in each quadrant.

For some of the series, exposure indices in addition to gravimetric dust concentration were measured. These are described in chapter 5, "Microbiology of the Fiber and Airborne Dust From Washed Cotton."

Control of Dust Concentration.—During the initial series of exposures, when subjects were exposed in the card room, interim dust concentration measurements were obtained from the average of four elutriator filters. The dust level could be adjusted upward or downward by one of several mechanisms. One of these was the card production rate, with higher production rates yielding higher dust levels. However, this parameter was established before exposure periods and not changed during exposures. The mechanism used to adjust the dust level was to change the local exhaust ventilation on the card machine, with more local exhaust being applied to decrease the dust concentration in the room. Air pickup points for the local exhaust system were located on the card at points where dust generation tended to be greatest.

For exposures in the remote room, the local exhaust system was removed. Card production rate and airflow through the card room were kept constant. A continuous aerosol monitoring system (ppm, Inc., Knoxville, Tennesse) was installed in both the card room and the remote room to give nearly instantaneous measurements of dust concentrations in both rooms. The dust concentration in the remote room was adjusted upward or downward by varying the proportion of card room exhaust air entering the exposure room. This was accomplished via a bleed-off valve located in the duct from the card room just prior to its entering the remote room.

Schedule of Exposures

Until 1982, exposures to cotton dust generally occurred every other day on 3 nonconsecutive days per week (usually Monday, Wednesday, and Friday) during periods of study. Clean room exposures were scheduled between the dust-exposure days (usually on Tuesdays and/or Thursdays). After early 1982, dust exposures were restricted to 2 days per week separated by at least 2 days (usually Monday and Friday), and clean room exposures (usually Wednesdays) were scheduled so as not to immediately follow a dust exposure day. This alteration in schedule was made because, although all previous data had indicated that baseline FEV_1 returned to normal by the day after exposure, it was believed that a schedule involving at least 2 days between dust exposures would be more analogous to the typical weekend respite from exposure experienced by textile mill workers. This scheduling of exposures was chosen

to limit the otherwise expected variability in acute ventilatory response related to the development of tolerance in cotton-dust-responsive individuals exposed on consecutive days to cotton dust (*8*).

Selection of Human Subjects

On several different occasions in the 5 years during which the washed cotton exposures were being conducted at Clemson, human subjects were selected for study. On each occasion, the selection process began with soliciting volunteers from the general public through newspaper advertisements, radio announcements, and word of mouth. The next step was to screen all volunteers with a questionnaire and spirometry to exclude those:

1) less than 18 or more than 65 years old;
2) with a history of asthma, chronic bronchitis, or other significant medical conditions that would preclude safe participation;
3) who regularly used medications (or had current jobs with exposure to agents) that affect airway response;
4) who had a FEV_1 less than 80 percent of predicted (*9*), using a correction factor of 0.85 for blacks (*2*).

Next, the remaining volunteers were exposed to card room cotton dust (at 1.0 mg/m^3 as measured by vertical elutriator) for 6-hour periods. Spirometry performed immediately before and after the 6-hour exposures enabled final selection of only those subjects who had an FEV_1 decrement of at least 5 percent attributable to cotton dust exposure. In the Clemson studies, approximately 30 percent of exposed volunteers had at least a 5 percent acute reduction in FEV_1 associated with these screening exposures (*10*). Cottons used for the screening exposures generally were white strict low middling Mississippi-grown cotton.

The study subjects were thus not a random selection from the general population. Similar to experimental human exposure studies of ambient air pollutants which use asthmatic individuals as sensitive indicators of acute effects of pollutants (*11*), the subjects were specifically selected to be relatively sensitive to the acute airways effects of cotton dust. However, to reduce the risk of participating in the experimental exposures, a small minority of particularly reactive individuals—those who experienced mean dust-related acute reductions in FEV_1 greater than 30 percent during the selection process—were excluded from participation.

The selection process was similar to that of Merchant et al. (*12*), except that those investigators selected their subjects from among active textile workers exposed to dust at their workplace, and not from volunteers from the general public exposed to controlled concentrations of cotton dust in an experimental cardroom.

Measurement of Cotton Dust Airway Activity

To measure the airway activity of cotton dust, each subject performed a series of forced expiratory maneuvers immediately before and after each 6-hour exposure. At each session, at least five blows were performed under the direction of a trained technician, using a waterless volume spirometer (Model 840; Ohio Medical Products, Madison, Wisconsin) directly interfaced with an oscilloscope to display flow-volume curves and an analog tape recorder to record flow volume curves. Initially these tapes were returned to the Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health (NIOSH). There the flow-volume signals were digitized and corrected for BTPS (body temperature, pressure, saturated with water vapor) for analysis. From the beginning of 1982, a computer (LSI-11; Digital Equipment Corporation, Maynard, Massachusetts) was directly interfaced with the spirometer, and flow-volume signals were directly digitized and BTPS corrected. All spirometric testing conformed to quality control requirements of the OSHA dust standard (*1*). Electrical and volume calibrations were consistently performed at the beginning and end of all spirometry sessions, both before and after exposures.

Although certain other spirometric parameters were also analyzed at various times during the course of the overall project, the FEV_1 was the parameter universally analyzed. The FEV_1 was selected as the parameter of most interest because it is relatively reproducible (*13*) and had been well established in a multitide of previous studies as an indicator of the airway effects of cotton dust inhalation. Furthermore, the FEV_1 parameter was judged to be relevant to the standard setting process because the 1978 OSHA cotton dust standard required medical surveillance of workers' FEV_1 responses to workplace exposures (*1*).

Results

Analysis of Data

Data from early series of exposures in the washed cotton project were analyzed slightly differently, but since 1981 the analysis of pulmonary function results has been the same for all exposure series. For analysis of dose response slopes, each subject's ventilatory function change and the gravimetric dust concentration for each exposure were considered. Simple linear regression analysis (which analysis of residuals had indicated was appropriate) was done for each experimental cotton using the following model:

$$\text{mean } \Delta FEV_1\ (\%) = \text{intercept} + \text{constant}_{dust} \times \text{gravimetric dust concentration.}$$

All results from the clean room exposures for a series of exposures were included in the regressions for each different cotton from that series. The slopes of these regression lines were considered to be a relevant parameter for describing the relative acute airway potency of each cotton dust. Thus, a dust devoid of acute airway activity should have a dose response slope that is no different from a slope of zero when tested for statistical significance using a one-sided *t*-test.

The results of the series of washed cotton exposures are most easily presented in chronological order. USDA study numbers are indicated to assist the reader in referring to other chapters in this monograph (Listings of all MQ numbers and wash treatments are given in apps. 1–3). Prior to the study of washed cottons, an earlier series of cotton dust exposures conducted jointly by USDA and NIOSH in 1978 had established that acute human exposure studies appeared to be a safe and useful method for determining effects of experiemental treatments for reducing the acute airway potency of dusts from various experimental cottons (*14*).

The first series of human exposures to washed (79–1D) and unwashed (79–1A) cottons at the Cotton Quality Research Station (79–1) was conducted during the summer of 1980 (*15,16*). A Mississippi-grown cotton was water-washed in lap form on a commercial rayon rinse system (see chapter 2). Human volunteers were then exposed on multiple, separate occasions to various concentrations of card-generated dust from the unwashed and the washed forms of this cotton, as well as to a clean room. Acute decrements in ventilatory function associated with exposure to dust from washed cotton were not significantly greater than those occurring on exposure to a clean room, in marked contrast to the effect of dust from unwashed cotton (table 4.1). The overall dose response regression analyses indicated that exposure to washed cotton at doses of up to 1 mg/m^3 resulted in an FEV_1 dose response relationship with no significant slope, whereas the dose response relationship for unwashed cotton dust had a highly significant slope (*16*).

A subsequent series of exposures (MQ–79) was intended to further the earlier experience of washing cotton on the rayon rinse system (*17*). In this study, cottons from California (MQ–79 C), Texas (MQ–79 E), and Mississippi (MQ–79 G) were washed. Results of this study indicated a major

Table 4.1—79–1 Exposure Results (32 Subjects; 6/80–8/80)

Treatment[a]	Exposures (*n*)	Dust levels (mg/m^3)	Dose response slope (Δ FEV_1 (%) per mg/m^3)	*p* (slope = 0)
79–1A	5	0.22–0.94	−5.9	<0.0001
79–1D	4	0.70–0.98	−1.0	NS

[a]Designations refer to cotton ID and washing treatment (see app. 2). A—Unwashed. D—Washed, rayon rinse system.
NS = Not significant.
Source: Table adapted from Boehlecke (*16*).

difference in the potency of conventionally harvested cottons in the unwashed form, with the California cotton (MQ–79 B) having much less acute airway activity than either the Mississippi (MQ–79 F) or Texas (MQ–79 D) cottons (table 4.2). Also, two water-to-cotton ratios (50:1 and 65:1 by weight: MQ–79 G and MQ–79 J, respectively, were used to assess the importance of this factor on the effectiveness of washing, but there were no significant differences in washing efficacy that could be related to the water ratio used (table 4.2). In addition, a preliminary trial of washing cotton (MQ–79 H) on a wool scouring system located in California was conducted. This preliminary trial system yielded cotton dust with a markedly reduced airway potency essentially equivalent to that washed on the rayon rinse system (table 4.2).

Washing cotton on a wool scouring system was further explored in another series of exposures (MQ–90). Although all various washing conditions yielded card-generated dusts with reduced acute airway activities, one of the lots (MQ–90 D) still retained dust with about half the potency of the unwashed cotton (MQ–90 A) (table 4.3). Other lots yielded results that were not significantly different from no effect, although there were consistent trends toward minimal residual activity. In addition, this series included exposure to dust from a washed cotton that had been stored for 7 months after washing (MQ–90 I, from MQ–79 C cotton). Results indicated that the acute airway potency of dust from washed cotton did not significantly increase during storage (table 4.3).

In a subsequent evaluation of cotton washed on the wool scouring system (MQ–91), even the best lot of washed cotton retained nearly half the potency of the unwashed cotton, and this was a statistically significant effect (table 4.4). An initial attempt to batch wash cotton (MQ–91 F) in a commercial system used for kier dyeing proved only minimally successful in reducing the acute airway potency of cotton dust, possibly because the cotton was not adequately wet out (table 4.4).

Further exposures to dust from cotton that was batch system washed in a kier (MQ–95) were carried out to explore the effects of wash temperature on the reduction of cotton dust potency. The overall mean results at both temperatures were equivalent (table 4.5). However, at high temperature (MQ–95 E, F, I, K, L), but not cold temperature (MQ–95 C, D, H), batch system washing resulted in a "cake" of cotton, with most residual activity located at the inflow side of the recirculating system. An important additional cotton (MQ–95 J) tested in this series of exposures was washed by a harsh method (very hot scour and bleach) on a continuous batt system at Cotton Incorporated, Greenville, South Carolina (see chapter 2 and app. 2). This cotton yielded little dust (0.39 mg/m^3 was the highest attainable level under the conditions used) and caused no significant airway response in the exposed subjects (table 4.5).

Table 4.2—MQ–79 Exposure Results (17 Subjects; 1/81–2/81)

Treatment[a]	Exposures (*n*)	Dust levels (mg/m^3)	Dose response slope (Δ FEV_1 (%) per mg/m^3)	*p* (slope = 0)
MQ–79 B	2	0.60–0.63	−2.8	NA
MQ–79 C	2	0.54–0.55	+1.3	NA
MQ–79 D	2	0.58–0.67	−5.4	NA
MQ–79 E	2	0.55–0.61	−0.9	NA
MQ–79 F	2	0.60–0.65	−8.5	NA
MQ–79 G	2	0.55–0.57	−0.9	NA
MQ–79 H	2	0.60–0.62	−1.4	NA
MQ–79 I	2	0.54–0.60	+0.2	NA
MQ–79 J	2	0.57–0.60	0.0	NA

[a]Designations refer to cotton ID and washing treatment (see app 2).
B—Unwashed.
C—Washed, rayon rinse system.
D—Unwashed.
E—Washed, rayon rinse system.
F—Unwashed.
G—Washed, rayon rinse system.
H—Scoured, wool scouring system.
I—Washed, rayon rinse system.
J—Washed, rayon rinse system.
NA = Not available.
Source: Table adapted from Petsonk et al. (*17*); *p* values not available.

Table 4.3—MQ–90 Exposure Results (18 Subjects; 8/4/81–8/21/81)

Treatment[a]	Exposures (*n*)	Dust level (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	*p* (slope = 0)
MQ–90 A	1	0.62	−5.9	<0.005
MQ–90 B	1	0.54	−1.4	NS
MQ–90 C	1	0.73	−6.6	<0.0005
MQ–90 D	1	0.59	−3.5	<0.025
MQ–90 E	1	0.64	−1.5	NS
MQ–90 F	1	0.64	−0.9	NS
MQ–90 H	1	0.58	−1.8	NS
MQ–90 I	1	0.53	+0.7	NS

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Washed wool scouring system, Lot B.
C—Unwashed.
D—Washed, wool scouring system, Lot 5.
E—Washed, wool scouring system, Lot 20.
F—Washed, wool scouring system, Lot 14.
H—Washed, wool scouring system, Lot 12.
I—Stored 7 months.
NS = Not significant.

Table 4.4—MQ–91 Exposure Results (14 Subjects; 9/14/81–10/2/81)

Treatment[a]	Exposures (*n*)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	*p* (slope = 0)
MQ–91 A	2	0.47–0.48	−9.0	<0.0005
MQ–91 B	2	0.37–0.50	−6.8	<0.0005
MQ–91 C	2	0.48–0.51	−3.6	<0.025
MQ–91 E	1	0.51	−7.0	<0.0005
MQ–91 F	2	0.30–0.48	−6.5	<0.0005

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Washed, wool scouring system, Lots 7, 8.
C—Washed, wool scouring system, Lots 4. 5.
E—Washed, wool scouring system, Lot 1.
F—Washed, batch kier system.

Table 4.5—MQ–95 Exposure Results (19 Subjects; 11/16/81–12/18/81)

Treatment[a]	Exposures (*n*)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	*p* (slope = 0)
MQ–95 A,B[b]	3	0.45–0.48	−7.9	<0.0005
MQ–95 C,D,H[b]	3	0.51–0.56	−2.1	<0.025
MQ–95 E,F,I,K,L[b]	4	0.48–0.51	−2.4	<0.01
MQ–95 J	1	0.39	+0.9	NS

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A, B—Unwashed.
C, D, H—Washed, batch kier system, 32 °C (90 °F).
E, F, I, K, L—Washed, batch kier system, 60 °C (140 °F).
J—Scoured and bleached, continuous batt system.
[b]Data from similar washing treatments are combined.
NS = Not significant.

A subsequent series of exposures (MQ–101) was done. In this series, exposure to dust from a harshly washed cotton (MQ–101 F, very hot scour and bleach on a continuous batt system) again yielded no significant airway response (table 4.6). All other washing treatments in this series of exposures gave variable results, with the batch system notably less effective than the continuous batt system (table 4.6).

Because of the low number of subjects (n = 12) involved in this MQ–101 series, washing on the Cotton Incorporated continuous batt system (with and without scour and bleach) was repeated with a large number of subjects (MQ–111) (*18*). In terms of acute airway potency, all three washings yielded markedly reduced potency, with response to washed-only cotton not significantly different from response to clean room exposure (table 4.7). Because of the effectiveness of washing in removing dust, the greatest exposure level for washed-only cotton was at 0.41 mg/m^3.

The earlier finding of no significant increase in potency for stored washed cotton was duplicated (MQ–111 D). Again, washed-only cotton did not increase in potency despite storage for 7 months (table 4.7).

Because we had become aware that commercially available cottons had varying potencies (*3, 19–21*), it was necessary to determine whether very potent cotton (i.e., cotton with high endotoxin) could be effectively washed (MQ–113). Depending on the specific wash conditions, potency was reduced by between 70 and 90 percent, although even in the latter case the minimal residual activity was still statistically significant (table 4.8).

In an attempt to further reduce the residual activity in dust from very potent cotton that was washed under mild conditions, an even more potent cotton (MQ–139) was washed with and without a continuous supply of fresh finish rinse. The airway potency of dust from washed-only cotton (MQ–139 C, table 4.9) was reduced by nearly 50 percent by the use of continuous fresh finish rinse (MQ–139 C, table 4.9). However, the addition of bleach to the wash water (MQ–139 D) was even more effective than the wash-only-treatment (table 4.9).

Table 4.6—MQ–101 Exposure Results (12 Subjects; 2/8/82—4/30/82)

Treatment[a]	Exposures (n)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	p (slope = 0)
MQ–101 A	2	0.41–0.53	−9.4	<0.0005
MQ–101 B	2	0.39–0.41	−4.7	<0.005
MQ–101 C	2	0.37–0.40	−2.7	NS
MQ–101 D	2	0.34–0.56	−1.4	NS
MQ–101 E	3	0.29–0.37	−2.9	<0.05
MQ–101 F	2	0.22–0.28	+0.4	NS
MQ–101 G	2	0.39–0.54	−3.3	<0.025
MQ–101 H	2	0.29–0.30	−0.4	NS
MQ–101 I	2	0.52–0.53	−5.0	<0.0005
MQ–101 J	2	0.41–0.45	−4.1	<0.005
MQ–101 K	2	0.46–0.58	−6.8	<0.0005

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Washed continuous batt system, 93 °C.
C—Washed, continuous batt system, 60 °C.
D—Scoured and rinsed, continuous batt system, 93 °C.
E—Washed and bleached, continuous batt system, 93 °C.
F—Scoured and bleached, continuous batt system, 93 °C.
G—Scoured and rinsed, continuous batt system, 60 °C.
H—Washed and bleached, continuous batt system, 60 °C.
I—Washed only, batch kier system, 60 °C.
J—Washed and bleached, batch kier system, 57 °C.
K—Scoured and rinsed, batch kier system, 60 °C.
NS = Not significant.

Table 4.7—MQ–111 Exposure Results

Treatment[a]	Exposures (n)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1(%) per mg/m^3)	p (slope = 0)
		55 Subjects; 6/14/82–10/1/82		
MQ–111 A	8	0.11–0.52	−7.8	<0.001
MQ–111 B	3	0.28–0.41	−0.2	NS
MQ–111 C	5	0.23–0.46	−1.0	<0.05
MQ–111 D	2	0.52–0.79	−1.2	<0.02
		56 Subjects; 4/4/83–4/8/83		
MQ–111 D[b]	4	0.36–0.55	−0.5	NS

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Scoured and bleached, continuous batt system.
C—Washed and bleached, continuous batt system.
D—Washed only, continuous batt system.
[b]Stored seven months after washing.
NS = Not significant.
Source: Table adapted from Cocke et al. (*18*).

Table 4.8—MQ–113 Exposure Results (47 Subjects; 1/17/83–6/10/83)

Treatment[a]	Exposures (n)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	p (slope = 0)
MQ–113 A	2	0.20–0.25	−26.6	<0.0005
MQ–113 B	2	0.42–0.51	− 6.2	<0.0005
MQ–113 B_2	2	0.32–0.35	− 9.2	<0.0005
MQ–113 H	2	0.40–0.46	− 3.2	<0.005
MQ–113 I	2	0.41–0.49	− 2.1	<0.025

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Washed only, continuous batt system.
B2—Washed only, continuous batt system.
H—Washed and bleached, continuous batt system.
I—Scoured and bleached, continuous batt system.

Table 4.9—MQ–139 Exposure Results (33 Subjects; 1/16/84–2/10/84)

Treatment[a]	Exposures (n)	Dust levels (mg/m^3)	Dose response slope (ΔFEV_1 (%) per mg/m^3)	p (slope = 0)
MQ–139 A	2	0.12–0.15	−50.8	<0.001
MQ–139 B	2	0.19–0.20	− 9.2	<0.01
MQ–139 C	2	0.23–0.25	− 5.5	<0.05
MQ–139 D	2	0.25–0.27	− 3.5	NS

[a]Designations refer to cotton ID and washing treatment (see app. 2).
A—Unwashed.
B—Washed only, continuous batt system, finish rinse recirculated.
C—Washed only, continuous batt system, finish rinse not recirculated.
D—Washed and bleached, continuous batt system, finish rinse not recirculated.

Summary Discussion

These human exposure studies have convincingly demonstrated that mild washing (essentially water rinsing) can reduce the acute bronchoconstrictor potency of residual dust in cotton. This elimination of the acute response is amplified by the fact that, in addition, this washing reduces the dustiness of cotton by at least 50 percent. Both effects seem to vary depending on the specific washing methods used as well as on the initial potency of the cotton washed. Dust from harshly (very hot scour and bleach) washed cotton proved on several different washing and exposure trials to be quite inert, as would be expected from earlier published reports (*12, 22*).

The rayon rinse system, when used to wash (with a water rinse) strict low middling Mississippi cotton, yielded consistently good results. The first washing study (79–1) concluded that the slope of the dose-response regression line did not differ significantly from zero, indicating no discernible effect of dust exposure. The next study (MQ–79) likewise indicated that exposure to dust from similar cotton washed on the rayon rinse system resulted in an acute effect that did not differ significantly from the clean room exposure effect. Washing conditions used are described in detail in chapter 2 and in appendix 2. No extremely potent cotton (cotton with high endotoxin) has been washed on this rayon rinse system.

Washing cotton on the wool scouring system yielded variable results. An initial trial of the wool scouring system (MQ–79) showed promise (table 4.2). However, one of the washed cottons in the first wool scouring system series of exposures (MQ–90) retained about half the potency of the unwashed cotton (table 4.3), and all wool scouring system washed cottons in the next series (MQ–91) retained measurable acute airway activity (table 4.4). Thus, it appears that this system, under the conditions used, is not optimal for cotton washing. Dust from batch system washed cottons, though less potent then unwashed cotton dust, also retained measurable acute airway activity, as demonstrated by trials in several series of exposures (MQ–91, MQ–95, and MQ–101; table 4.4, 4.5, and 4.6).

Dust from strict low middling cotton washed on the Cotton Incorporated continuous batt system yielded dust with a dose-response slope not different from zero. Also washed on the continuous batt system were very potent cottons. As the results of the MQ–113 series indicate (table 4.8), measurable acute airway activity remained in this exceptionally potent cotton even after washing. However, the addition of bleach or bleach and an alkali scour resulted in a significant further reduction of cotton potency over and above the substantial reduction resulting from washing alone. In addition, it was demonstrated in the MQ–139 series (table 4.9) that the wash can be improved by using a continuous supply of fresh finish rinse, rather than allowing contaminants to build up in the finish bath.

All of these studies were limited to investigating only the acute effects of cotton dust inhalation in a select population under experimental conditions. We cannot, with complete confidence, presume that cottons shown to have minimal acute activity will pose minimal risk for chronic airways obstruction when inhaled over a working lifetime in the mills. This relationship of acute and chronic airway effects is plausible, but only a long-term prospective study could objectively document it.

A major problem with making explicit recommendations for a washed cotton standard to allow mild washing derives from our studies of "worst case" cotton with high endotoxin levels (MQ–113 and MQ–139; see app. 2). Washing extremely potent cotton with water alone yielded cotton dust that retained approximately as much acute toxicity as typical unwashed strict low middling cotton. Thus, the same permissible exposure level should be applied to mildly washed "worst case" cotton as is currently in place for cotton dust in general. Additional treatment of the cotton by bleaching or by scouring and bleaching (as in MQ–113 H, MQ–113 I, and MQ–135 D) reduced the potency of resulting dust to less than 50 percent of a standard cotton dust (see MQ–111 A).

Ultimately, it would be desirable to have a reliable laboratory test that would be more indicative of the acute and chronic pulmonary toxicity of cotton dust, both washed and unwashed, than the gravimetric elutriated dust measurement. Investigations of such a predictive test or tests have been conducted in conjunction with the washed cotton studies. Being explored are potentially useful indicators using microbial analyses, both for viable organisms (*20*) and for endotoxin (*3, 21, 22*), as well as chemical analyses (*23*).

Recent observations describe the highly correlated relationship between airborne endotoxin concentration and acute ventilatory function changes in subjects exposed to dusts from a variety of unwashed cottons (*3*; Castellan, R. M. et al., unpublished observations). From these quantitative dose-response relationships, it is conceivable that a standard based on endotoxin exposure may eventually replace the current permissible exposure limit based on gravimetric dust.

Given the observations generally presented in this chapter and the generally marked reduction of endotoxin that results from washing (see chapter 5), it is reasonable to suggest that cotton that has been effectively washed in terms of residual endotoxin could be justifiably exempted from the current gravimetric dust permissible exposure limit. However, until more is known about the effectiveness of washing as it relates to the prevention of the chronic obstructive ventilatory impairment associated with cotton dust inhalation, prudent health practice should continue to include serial medical surveillance of workers exposed to dust from washed cotton.

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Chapter 5
Microbiology of the Fiber and Airborne Dust From Washed Cotton

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Introduction

Cotton fibers were shown by light microscopy to be contaminated with gram-negative bacteria (*1*). Electron microscopic examination verified the intimate association of bacteria and the cotton fiber and demonstrated additionally the peeling of the outer bacterial membrane and release of endotoxin (*2, 3*). Endotoxins are lipopolysaccharide-protein complexes (*4*) which have the potential to exert profound biological effects in vivo and in vitro (*5, 6*).

Experimental exposures of laboratory animals to inhaled bacteria and their endotoxins resulted in functional and histologic alterations in the lungs (*7, 8–12*). Workers who are exposed occupationally to airborne endotoxin-containing dusts in swine confinement buildings (*13, 14*) or in sewage treatment plants (*15*) exhibit signs and symptoms such as chest tightness, cough, excess sputum/phlegm, nasal irritation, shortness of breath, wheeze, and fever. Human and experimental animal data have shown that the lung serves not only as a portal of entry for airborne bacteria and endotoxins but as a site for localized tissue, cellular, and functional alterations as well.

Removal or reduction of the gram-negative bacterial and endotoxin burdens in the cottons and their related dusts would provide the opportunity to evaluate the role these agents may play in eliciting respiratory pathophysiology in cotton workers. Washing bulk cottons before the carding process is one method of accomplishing this goal. Treatment of card room cotton dust by physical and chemical protocols that paralleled the commercial preparation of medical grade cotton resulted in the removal of over 98 percent of the endotoxin activity in the dust (*16*). Experimental challenge of laboratory animals with the treated card room dust resulted in only minor alteration of pulmonary function as monitored by arterial blood gas levels when compared with similar exposures with the untreated dust (*17*). The laboratory precendents indicate, therefore, that washing of cottons may be an effective method for removing gram-negative bacterial endotoxins, thereby reducing the acute pulmonary function effects caused by inhaling contaminated dusts.

As part of the trials undertaken by the Task Force for Byssinosis Prevention, samples of unwashed and washed lints and of airborne dusts were collected when cottons washed on each washing system were carded. These samples were evaluated for different microbiological characteristics including total bacteria, gram-negative bacteria, fungi, actinomycetes, and endotoxins. Relationships between the microbial content of the unprocessed fiber and the microbial content of the card-generated dust are examined. Different washing treatments as well as different sources and types of cottons are discussed within the context of viable microorganisms and endotoxins.

Materials and Methods

Test Cottons

The majority of cotton used for the washing trials was machine-picked, DPL–61 grown in Mississippi in 1980. The cotton was shipped to the USDA, Agricultural Research Service's Cotton Quality Research Station at Clemson, South Carolina, where 48-bale lots were opened, blended, and then rebaled to assure as much homogeneity between bales as possible. This cotton was designated as MQ–80 cotton. Additional information about this fiber is given in chapters 2 and 3 and appendix 2.

A second cotton was evaluated in 1982. This cotton was also grown in Mississippi but was of lower grade and was selected specifically on the basis of the microbiological contamination of the lint. A 25-bale lot was purchased and shipped to the Cotton Quality Research Station where it was blended and rebaled. This cotton was designated as MQ–113.

Other cottons that were used during some of the trials included small lots of commercially available cottons grown in Texas, Mississippi, and California. Bulk lint samples and airborne samples from the exposure room were collected for cotton treatments MQ–79, 101, 107, 111, 113, and 139 (see appendix 2 for a description of the specific treatment for each cotton). Only bulk lint samples were collected for treatments MQ–89, 90, 91, and 95.

Washing Systems

The washing systems used in these trials are outlined in chapter 2.

Fiber and Water Samples

Most samples of fiber were collected after drying for each treatment. Gram quantities of lint were placed in a paper sack and shipped to the laboratory for microbiological analysis and endotoxin evaluation. If wet samples were collected, they were either sealed in plastic bags and stored at 4 °C until analysis or dried in a convection oven at 50 °C and treated as a dried sample.

Water samples were collected in pyrogen-free tubes and stored at 4 °C until analysis. For most wet lint and water samples, analysis was done within 48 hours of collection of the sample.

Microbiological Analysis

The following procedures were used in the microbiological analysis of fiber and water samples collected during each washing trial.

Specimens of cotton, each weighing 3 g, were suspended in 25 or 50 ml of pyrogen-free water, vortexed for 5 minutes, and subjected to low power sonication on a sonic cleaner for 10 minutes. Control studies showed that mild sonication did not reduce bacterial counts and enhanced release of bacteria and endotoxin from fiber. Serial 10-fold dilutions in pyrogen-free water were made, and 0.1 ml (or an appropriate amount) of each dilution was spread evenly over the surface of a plate. The media used were trypticase soy agar (TSA) with cycloheximide (0.4 g/l) for gram-positive (GPB) and total bacterial counts, and TSA with cycloheximide and vancomycin (15 mg/l) for gram-negative bacterial counts, and Jebauraud agar with NaCl (1.1M) for fungi. Plates were incubated at 25 °C for 1-2 weeks and colonies were counted. Total bacterial counts were made on plates incubated at 37 °C. Calculations give the number of colony-forming units (CFU) per gram of cotton. Each sample was assayed in triplicate, and results were reported as a mean value. For samples in which no organisms were detected at the lowest dilution, the values were reported as less than the lowest level detectable at that dilution.

Endotoxin was measured by the *Limulus* amebocyte lysate (LAL) test as modified by Kreeftenberg et al. (*18*). Twenty µl of each of four or six serial 10-fold dilutions of the original extract were tested in a flat-bottomed microtiter plate containing 20 µl of LAL per well and incubated at 37 °C for 60 minutes. Ten µl of 0.2 percent toluidine blue were added and gelation was read by tipping the plate after 5 minutes. Results were calculated as nanograms endotoxin activity per gram of cotton or per cubic meter of air if airborne dusts were studied. Endotoxin activity was defined on the basis of the titer of a control endotoxin (*Escherichia coli*, #0113, PPE-E-434, supplied by Associates of Cape Cod, Inc., Woods Hole, Massachusetts). Therefore all of the data are expressed relative to the *Limulus* activity of *E. coli* endotoxin. Biological activity of endotoxin from GNB (gram-negative bacteria) found on both lint and airborne dust samples of cotton may be quite different from the activity associated with *E. coli* endotoxin.

Enumeration of Bacteria and Actinomycetes on Lap and Bale Samples

The cotton extract and gram-negative selection media described in the next section, "Airborne Viable Microorganisms," with the omission of agar, were used in a microdilution MPN (most probable number) method (*19*) for enumeration of the total and gram-negative bacterial content of bale and lap fiber. Bale samples were composited from five subsamples of a bale after opening but before blending. Lap samples corresponded to those carded during the air sample periods. Composite 1.0 g random subsamples for each lap or bale were wetted in pH 7.2 0.5 M phosphate-buffered water and shaken for 3 hours at 10 °C. The primary dilution was adjusted according to the anticipated level of bacterial content; the lowest possible dilution was 1:25 (fiber: buffer) for scoured-and-bleached samples. Dilutions of fiber from the unwashed, low-dust-level treatment were made directly into CE (cotton extract) and GNS (Gram Negative Selection) broths. Dilutions for all other samples were allowed a 24-hour enrichment phase in CE broth before being replica-plated via droplet inoculation into GNS broth. Dilutions in the 96-well microtiter plates were incubated at 25 °C, and results were recorded at 24 hours for total bacteria and at 48 hours for gram-negative bacteria. Results were computed as $\log_{10}$ MPN/g of fiber.

Thermophilic antinomycetes of the genus *Thermoactinomyces* were enumerated by first plating five randomly selected wisps of cotton (approximately 20 mg per wisp) directly onto Thermoactinomycete Selective medium (CYC agar) and counting characteristic colonies that grew after 24 hours at 50 °C. Samples that had more than 30 colonies per plate were dilution plated (in duplicate), incubated, and counted as described.

Data were stored and analyzed at the USDA Washington Computer Center, Washington, D.C. Treatment means were compared using Duncan's multiple range test. Linear regression procedures were used to analyze the relationship of the amounts of microorganisms in the airborne dust to their amounts on the corresponding uncarded lint; and the size distribution of airborne particles bearing microorganisms. The latter was accomplished by using the probit function of the cumulative proportion of the particles at each stage of the sample. Log transformation of the lower size limit of each stage was used as the size value.

Airborne Viable Microorganisms

Air samples for analysis of viable microorganisms were obtained with the six-stage viable particle Andersen air sampler (Andersen 2000 Inc., Atlanta, Georgia) operated at a flow rate of 0.028 m^3/min. Two samples were stationed in each exposure room at positions corresponding to the positioning of vertical elutriators (VE). The orifice of each Andersen sampler was upright and at the approximate height of the VE orifice. Refer to appendix 5 or chapter 4.

Samplers were loaded with 90 mm plastic petri plates filled with 30 ml of water agar overlayed with 10 ml of appropriate nutrient agar. This amount of agar was necessary in

order to bring the top surface of the agar in each plate to the prescribed distance from the sieve at each stage of the sampler, thereby preserving the particle sizing characteristics of the sampler (*20*). For treatments MQ–107 and MQ–111, air samples were impacted directly onto media that are selective for total and gram-negative bacteria. For treatments MQ–113 and MQ–139, air samples for total bacteria were impacted directly onto the medium (described below) for total bacteria, after incubation for 24 hours at 25 °C, colonies were replica-plated onto the gram-negative selecton agar (described below). For total bacteria, cotton extract agar (CEA) with the following composition was used (grams): $(NH_4)_3PO_4$, 0.5; K_2HPO_4, 0.5; $MgSO_4$ $7H_20$, 0.2; NaCl, 1.0; yeast extract (Difco, Detroit, Michigan), 5.0; glucose, 5.0; agar, 20.0. Cotton extract was added and the volume was increased to 1 l with distilled water. Cotton extract was obtained by soaking 10 g of unwashed cotton in 1000 ml of distilled water at room temperature for 0.5 hours, hand-expressing the liquid, and filtering it through several layers of cheesecloth. Cycloheximide (Sigma Chemical Co., St. Louis, Missouri) was added to cooled (55 °C) media to achieve a final concentration of 50 μg/ml. For gram-negative bacteria, gram-negative selection agar (GNSA) with the following composition was used (grams per liter of distilled water): peptone, 5.0; glucose, 1.0; K_2HPO_4, 0.03; sodium lauryl sulfate, 0.5; bromthymol blue, 0.03; agar, 20.0. Cycloheximide was added as described above. For fungi, glucose yeast extract agar (GYEA) with the following constituents was used (grams per liter of distilled water): glucose, 5.0; yeast extract, 5.0; peptone, 5.0; agar, 20.0; gentamicin sulfate (Sigma Chemical Co.), 0.1.

For Thermophilic actinomycetes, the thermoactinomycete selective medium (CYC) of Cross and Attwell (*21*) was used. Plates were incubated at 50 °C for 24 hours and colonies counted.

For all groups of microorganisms studied, the amounts of particles bearing bacteria (PBB) or fungi (PBF) in airborne dust were calculated according to the expression:

$$PBB/m^3 = \text{(Total number of colonies on 6 stages) X } (0.028\ m^3/\text{min.}) \text{ X (min. sampled).}$$

Sample times were adjusted (prior to the start of each test) so that each of the individual stages of the sampler had less than 400 colonies, and preferably less than 200 colonies. Sample times ranged from 0.5 to 5 minutes for unwashed cotton and 25 to 45 minutes for washed, bleached, or scoured cotton.

One-hour samples were collected on days when no cotton was processed and consequently no dust exhausted into the exposure rooms (designated "clean room").

Endotoxin Analyses of Airborne Dust Samples

During the exposure trials with MQ–79 treatment cottons, dust samples were obtained by hanging personal air samplers on the human subjects near their breathing zones. Five μm pore size, 37 mm polyvinyl chloride filters (VM–1, Gelman Sciences, Inc., Ann Arbor, Michigan) were used to collect the dust in open face cassettes attached to calibrated model G pumps (Mine Safety Appliances Co., Pittsburgh, Pennsylvania) during the 6-hour exposures.

Samples of airborne dusts that were generated during the carding of cottons from treatments designated MQ–107, 111, 113, and 139 were obtained from vertical elutriators (VE) which were positioned in different areas of the exposure rooms. Dusts were collected on 5 μm pore size, 37 mm filters as was done in the MQ–79 trials.

All filters were labeled by number, and no reference to exposure, treatment of the cottons, or area of growth of the cottons was made until after the endotoxin analyses were completed. Sterile, nonpyrogenic plasticware was used throughout the laboratory procedures. Each filter was extracted separately with 10 ml sterile, nonpyrogenic water (Travenol Laboratories, Inc., Deerfield, Illinois) by rocking in a screw-capped 50 ml centrifuge tube at room temperature for 60 minutes. The extract fluids were centrifuged at 1000Xg for 10 minutes to remove particulate debris, and the supernatant fluids were separated. Analyses were performed immediately, or the fluids were frozen and stored at −80 °C. Extracts from the personal sampler filters from MQ–79 were assayed separately, while 5 ml aliquots from 2 to 5 VE filter extracts, which represent dust that was collected during the same time period in the same exposure room, were combined for analysis.

Gram-negative bacterial endotoxin contents were quantified in duplicate by a spectrophotometric modification of the *Limulus* amebocyte lysate gel test (Pyrostat; Millipore Corp., Bedford, Massachusetts). The lysate of the amebocytes from the horseshoe crab, *Limulus polyphemus*, clots in the presence of endotoxins (*22*), and the resulting turbidity is quantified spectrophotometrically by measuring an apparent increase in absorbance at 360 nm. Results were analyzed by linear regression, compared to a standard curve, and reported in terms of nanograms of U.S. Reference Endotoxin per milligram of airborne cotton dust or per cubic meter of air.

Air samples were collected during each trial period when no cottons were carded. The filters from those days were designated "clean room" and used as negative controls. Each clean room filter was treated similarly to the test filters except that each filter was assayed for endotoxin content separately.

Results of Lint Analysis

Rayon Rinse System

The first cottons washed on the rayon rinse system were from three different areas of growth; they were evaluated for the effect of water temperature and water-to-fiber ratios on removal of biological activity. The unwashed cotton from Texas had the highest levels of microbiological contamination followed by cottons grown in Mississippi, then California. The effects of different water-to-fiber ratios and water temperatures on removal of GNB are shown in table 5.1. Each of the cottons was washed at 66 °C and a 50:1 water-to-fiber ratio. The levels of GNB were reduced by approximately 1 log for all three cottons; however, only the more heavily contaminated Texas and Mississippi cottons showed a measurable reduction of endotoxin. The levels of endotoxin on the Mississippi and Texas cottons were reduced from 800 to 80 ng/g by the washing treatment. The initial level of 80 ng/g on the California cotton was not changed by washing. Endotoxin levels and GNB levels from Mississippi cotton washed at different temperatures (66 °C vs. 28 °C) or at different water-to-fiber ratios (50:1 vs. 65:1) were similar.

Wool Scouring System

The second system used for washing was a commercial wool scouring facility. Preliminary evaluation of two washing temperatures, 27 °C and 49 °C, showed reductions of 1 log in GNB for both temperatures (Study MQ–89, data not included). The removal of lint-associated endotoxins was highly variable, with as much as 50 percent of the original endotoxin remaining associated with the washed and dried fiber. In general, the higher washing temperature was more effective in reducing the viable bacteria and endotoxin from the lint. A second trial using MQ–80 cotton and designated as Study MQ–90 was designed to evaluate the effect of water-to-fiber ratios on reduction of biological activity. The results (table 5.2) show that both water-to-fiber ratios tested were equally effective in reducing viable GNB and endotoxin. The levels of viable GNB were reduced by over 3 logs by each treatment; however, washing reduced the levels of endotoxin by less than 1 log.

Table 5.1—GNB on Cottons Washed on the Rayon Rinse System (MQ–79)

Area of growth	Temperature	Water-to-fiber ratio	GNB (log_{10} CFU/g)[a]		Change (%)[c]
			Unwashed[b]	Washed[b]	
California	66 °C	50:1	4.72 (MQ–79 B)	3.58 (MQ–79 C)	92.9
Texas	66 °C	50:1	6.20 (MQ–79 D)	3.60 (MQ–79 E)	97.8
Mississippi	66 °C	50:1	5.07 (MQ–79 F)	4.08 (MQ–79 G)	89.8
Mississippi	66 °C	65:1	—	3.37 (MQ–79 J)	98.0
Mississippi	28 °C	65:1	—	3.85 (MQ–79 I)	93.9

[a]Means of triplicate determinations.
[b]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[c]Percent change in viable bacteria.

Table 5.2—Effect of Different Water-to-Fiber Ratios on Removal of GNB and Endotoxin From Mississippi Cotton Washed on the Wool Scouring System (MQ–90)

Lot and treatment[a]	Water-to-fiber ratio	*n*	GNB (log_{10} CFU/g)[b]	Endotoxin (ng/g)[b]
1 (MQ–90 B, D)	40:1	5	2.60 ± 0.17	76 ± 36
2 (MQ–90 E, F, H)	20:1	8	2.51 ± 0.21	85 ± 30
Unwashed (MQ–90 C)	—	6	5.90 ± 0.49	620 ± 10

[a]Washed at 49 °C. Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Mean ± standard error.

A third trial (MQ–91) was designed to evaluate a higher washing temperature (60 °C) and water-to-fiber ratios of 20:1 and 40:1. This trial used the MQ–80 cotton. Before this experiment, the wool scouring line was scrubbed with a detergent and then rinsed with a hypochlorite solution. Samples of lint were collected after washing but before drying for microbiological analysis (table 5.3). These samples were dried overnight in a convection oven at 50 °C. The levels of GNB and endotoxin on the unwashed lint ranged from 2–8 $\times$ 10^5 CFU/g and 440–800 ng/g respectively. During the trial, fresh water was only added after the water-to-fiber ratio was established or changed and when water was needed to replace fiber-associated water removed from the final bowl before drying. The GNB predried counts showed a reduction of > 3 logs for lots 1–3. However, as the trial progressed, there was a measurable increase in bacteria on the washed fiber (predried lots 4–8). This was observed in the dried as well as in the predried samples, although the high drying temperatures (120 °C for 10 minutes) did reduce the viable population of GNB on the dried samples. A similar trend was observed for endotoxin. The three initial lots of cotton showed reduced levels of endotoxin after which a progressive increase occurred during the washing trial. Samples of water collected from each of the five bowls were evaluated for endotoxin and GNB (table 5.4). The source water contained < 10 ng/ml of endotoxin and < 10 CFU/ml GNB. The first water sample was collected immediately after the bowls were filled and showed no elevation in levels of

Table 5.3—Microbiological Analysis of Cotton Washed on the Wool Scouring System (MQ–91)

Date and treatment[a]	Water-to-fiber ratio	GNB (log_{10} CFU/g)[b]			Endotoxin (ng/g)[b]		
		Unwashed[c]	Predried	Dried	Unwashed[c]	Predried	Dried
9/10, lot 1	>40:1	5.71	1.60	2.15	440	4	22
9/10, lot 2	>40:1	5.30	2.04	1.90	800	40	40
9/10, lot 3	>40:1	5.36	2.34	1.90	440	40	40
9/11, lot 4 (MQ–91 C)	40:1	6.94	2.65	1.90	800	400	40
9/11, lot 5 (MQ–91 C)	40:1	5.81	3.34	2.78	800	400	220
9/11, lot 6	>20:1	5.30	4.89	2.08	440	400	400
9/11, lot 7 (MQ–91 B)	20:1	5.30	5.04	2.30	800	400	400
9/11, lot 8 (MQ–91 B)	20:1	5.30	3.30	2.04	800	400	400

[a]Fiber washed at 60 °C. Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Values are means of triplicate determinations.
[c]Unwashed values are for MQ–91 A.

Table 5.4—Effect of Time on the Level of GNB and Endotoxin in the Water Baths of the Wool Scouring System (MQ–91)

Date	Sample	Washing bowl[a]									
		1		2		3		4		5	
		GNB	LPS	GNB	LPS	GNB	LPS	GNB	LPS	GNB	LPS
9/9[b]	1	<10	5	<10	5	<10	5	<10	0.5	<10	5
9/10[c]	2	2.5 $\times$ 10^4	50	280	5	<10	5	220	5	1.7 $\times$ 10^3	5
9/10[d]	3	<10	50	<10	50	290	50	700	50	1.8 $\times$ 10^3	50
9/11[e]	4	<10	50	510	500	50	50	<1 $\times$ 10^6	50	2.4 $\times$ 10^5	50
9/11[f]	5	<10	50	<10	50	<10	50	6.4 $\times$ 10^5	50	7.7 $\times$ 10^5	500

[a]GNB = CFU/ml; LPS = endotoxin ng/ml; means of triplicate determinations.
[b]Fresh input water—room temperature.
[c]Water in bowls overnight—room temperature.
[d]Water in all bowls at 60 °C.
[e]Water in bowls overnight; cooled to room temperature.
[f]Water in bowls 1–3 at 60 °C; bowls 4 and 5 at room temperature.

GNB or endotoxin. The second sample was collected after the water was held in the bowls overnight but before washing. For this sample the levels of GNB increased in four of the bowls, and endotoxin increased in one of the bowls. Because sample 2 was collected before processing of the cotton, the increases in GNB and endotoxin apparently are results of growth from residual contaminants in the system. After sample 2, all bowls were heated to 60 °C, and the washing process was started. Sample 3 was collected at the end of the washing period for day 1. Lint samples designated lots 1–3 (table 5.3) were collected during the first day of washing. The data show an increase of viable bacteria and endotoxin in the final two bowls even though the temperature of the wash water was 60 °C. For the final day (samples 4 and 5), only bowls 1 through 3 were heated to 60 °C. This apparently limited the growth of bacteria in these bowls; however, in bowls 4 and 5, the levels of both GNB and endotoxin continued to increase. The increase of endotoxin and GNB in the final bath probably accounts for the increase on the dried lint (lots 4–8, table 5.3). Although the fiber had been washed and rinsed, passage through the final bowl may have recontaminated the fiber with both viable bacteria and endotoxin.

Batch Kier System

The third system used for washing was the batch kier system. This system differed from the others in that the cotton was processed in discrete batches rather than through a continuous process. MQ–80 cotton was used for all washing studies. The first trial evaluated the effect of different temperatures, 49 °C and 60 °C, on washing efficiency (table 5.5). Both dried and predried samples were collected for evaluation of GNB and endotoxin. For this trial, predried samples were not dried but stored on ice and returned to the laboratory for analysis. The levels of GNB were reduced by more than 2 logs and endotoxin by more than 1 log for all samples at each temperature. Both temperatures were equally effective in reducing the levels of GNB and endotoxin on washed fiber. Because there was no apparent difference in the effect of these two temperatures on washing efficiency, a second trial (MQ–95) was done to evaluate a lower washing temperature. In the batch kier system, water is circulated through the "cake" of fiber from the top to the bottom and then forced out with compressed air. Because the top of the cake is drier than the bottom, predried samples were collected from different levels of the cake to determine if there was an accumulation of GNB or endotoxin at a particular level. The data in table 5.6 show that for each trial both endotoxin and GNB were reduced from unwashed levels in lot 1; however, the predried samples indicate that there was an accumulation of GNB in the bottom layer. This may be a reflection of the higher percentage of residual washing water retained in the less-well drained bottom layers of the cake. The levels of both endotoxin and GNB were higher in the fiber washed at 32 °C. These data suggest that for this system there is a washing temperature threshold below which the removal of GNB and endotoxin becomes less efficient. After the fiber is dried at 121 °C for 7.5 minutes, the levels of GNB were reduced by 2 logs from prewashed samples and endotoxin by less than 1 log for all trials.

A third trial (MQ–101) was done to evaluate the effects of more severe washing treatments on removing acute human reactivity. Cottons were either washed, washed and bleached, or scoured and rinsed. The levels of GNB and endotoxin for each treatment are shown in table 5.7. For endotoxin, the more severe wash-and-bleach and scour-and-rinse treatments resulted in larger reductions of endotoxin on the fiber. The levels of GNB on washed fiber were reduced by 2 logs for all treatments.

Table 5.5—Effect of Temperature on GNB and Endotoxin Levels on Cotton Washed on the Batch Kier System (MQ–91)

Treatment[a]	GNB (log_{10} CFU/g)[b]			Endotoxin (ng/g)[b]		
	Unwashed[c]	Predried	Dried	Unwashed[c]	Predried	Dried
60 °C (MQ–91 F lot 1)	5.00	2.00	<1.9	440	40	40
60 °C (MQ–91 F lot 1)	4.75	<2.00	<1.9	80	40	40
49 °C (MQ–91 F lot 2)	6.00	<2.00	<1.9	440	40	40
49 °C (MQ–91 F lot 2)	4.60	<2.00	<1.9	440	40	40

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Means of triplicate determinations.
[c]Unwashed values are from MQ–91 A.

Table 5.6—Effect of Temperature on GNB and Endotoxin Levels on Cotton Washed on the Batch Kier System (MQ–95)[a]

Sample	Lot 1[a] GNB[c]	Lot 1[a] Endotoxin[d]	Lot 2[b] GNB[c]	Lot 2[b] Endotoxin[d]
	MQ–95 A[e]			
Unwashed				
Bale 1	4.46	800	4.50	800
Bale 2	4.81	800	4.23	800
	MQ–95 K, L[e]		MQ–95 C, D, H[e]	
Predried				
Top	1.30	40	6.00	400
Middle	ND	ND	7.28	400
Bottom	3.39	220	7.30	400
Dried[f]				
Bale 1	1.60	40	2.63	40
Bale 2	1.30	40	1.30	40
Bale 3	1.30	40	1.30	40
Bale 4	2.00	40	NS	NS

[a]Lot 1 washed at 60 °C.
[b]Lot 2 washed at 32 °C.
[c]Log_{10} CFU/g; means of triplicate determinations.
[d]ng/g; means of triplicate determinations.
[e]Designations refer to cotton ID and washing treatment (see app 2).
[f]2 unwashed bales were rebaled into 3 or 4 washed bales.

Table 5.7—Effect of More Severe Washing Conditions on the Levels of GNB and Endotoxin on Cottons Washed on the Batch Kier System (MQ–101)

Treatment[a]	GNB (log_{10} CFU/g)[b] Unwashed[c]	GNB (log_{10} CFU/g)[b] Washed	Endotoxin (ng/g)[b] Unwashed[c]	Endotoxin (ng/g)[b] Washed
Wash only[d] (MQ–101 I)	4.84	1.91	4.6×10^3	420
	4.96	1.32	4.6×10^3	420
	—	1.62	—	420
Wash and bleach (MQ–101 J)	5.00	1.80	4.6×10^3	23
	4.52	1.32	8.3×10^2	42
	—	2.64	—	>42
Scour and rinse (MQ–101 K)	4.83	1.32	4.6×10^3	42
	5.41	2.20	8.3×10^3	>42
	—	2.88	—	420

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Means of triplicate determinations
[c]Unwashed values are from MQ–101 A.
[d]Washed at 60 °C. 2 bales unwashed rebaled into 3 bales washed.

Continuous Batt System

During the trials of the batch kier system, samples of scoured and bleached fiber, processed on a modern continuous batt system, were evaluated for biological activity. Human panel responses to this cotton were not significantly different from acute responses to no exposure (chapter 4); therefore additional washing trials were planned to evaluate the continuous batt system for washing cotton.

The results of the first trials using MQ–80 cotton and designated as MQ–101 are summarized in table 5.8. Two temperatures and four washing treatments (wash only, bleach only, scour only, and scour and bleach) were evaluated and subsamples collected for microbiological analysis. After drying, samples from all treatments showed reductions of > 2 logs for GNB and at least 1 log for endotoxin.

Based on the human panel response to Study MQ–101, three washing treatments were selected for large-scale trials in which approximately 680 kg of MQ–80 cotton were processed for each treatment. The results of the microbiological analysis of lints from each of these trials (MQ–111) are shown in table 5.9. For all trials, GNB levels on dried lint were reduced by > 3 logs and endotoxin by 1 log. Human panel evaluations of these cottons showed that the wash-only treatment removed the acute biological activity from the cotton (chapter 4). Cotton washed by the scour-and-bleach and wash-and-bleach treatments retained a small but significant portion of the acute biological activity. The retention of activity by the more severely treated cottons was not readily explained by any of the measured microbiological parameters.

Most of the washing trials on the wool scouring system, the batch kier system, and the continuous batt system used the MQ–80 cotton. This cotton is typical of that normally used in a textile mill; however, there are bales of cotton available that are heavily contaminated with microorganisms and related metabolites. Because of the success in removing the biological reactivity and microbiological contaminants from MQ–80 cotton using the continuous batt system, a trial (MQ–113) was designed in which a cotton heavily contaminated with microorganisms was washed. The cotton used for this trial was also designated MQ–113. Several bales of this type of cotton were purchased, evaluated microbiologically, and treated by wash only, wash and bleach, and scour and bleach on the continuous batt system. The levels of microbiological contamination are given in table 5.10. Prewashed levels of GNB and endotoxin were 1 log higher than levels on MQ–80 cotton. For all washing treatments, the levels of GNB, gram-positive bacteria (GPB), fungi, and endotoxin were reduced by several logs; however, the levels of endotoxin remaining on the washed fiber were substantially higher than levels for the MQ–80 cotton washed by similar treatments. None of the

Table 5.8—Effect of Different Temperatures and Washing Conditions on the Levels of GNB and Endotoxin on Cotton Washed on the Continuous Batt System (MQ–101)

Treatment[a]	GNB (log_{10} CFU/g)[b]			Endotoxin (ng/g)[b]		
	Unwashed[c]	Predried	Dried	Unwashed[c]	Predried	Dried
Wash only						
93 °C (MQ–101 B)	5.04	TNTC	≤1.32	833	2292	136
60 °C (MQ–101 C)	4.95	TNTC	2.72	833	2292	42
60 °C (MQ–101 C)	4.80	NS	≤1.62	833	NS	42
Wash and bleach						
93 °C (MQ–101 E)	5.68	NS	1.80	8333	NS	417
93 °C (MQ–101 E)	4.87	2.72	≤1.32	833	42	230
60 °C (MQ–101 H)	4.98	NS	<1.32	833	NS	42
Scour and rinse						
93 °C (MQ–101 D)	5.15	NS	2.62	8333	NS	42
93 °C (MQ–101 D)	4.97	1.62	1.92	833	42	136
60 °C (MQ–101 G)	4.80	NS	≤1.92	833	NS	230
Scour and bleach						
93 °C (MQ–101 F)	5.26	NS	1.86	833	NS	417
93 °C (MQ–101 F)	5.00	NS	<1.12	833	NS	42

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Means of triplicate determinations.
[c]Unwashed values are from MQ–101 A.

Table 5.9—Large-Scale Evaluation of Effects of Different Washing Treatments on GNB and Endotoxin Levels On Cotton Washed on the Continuous Batt System (MQ–111)

Treatment[a]	GNB (log_{10} CFU/g)[b]			Endotoxin (ng/g)[b]			
	n	Unwashed[c]	Dried	*n*	Unwashed[c]	Dried	(%)[d]
Wash only (MQ–111 D)	10	4.51 ±0.50	≤1.32	15	$1.2 \times 10^3 \pm 317.0$	134 ±36	88.7
Wash and bleach (MQ–111 C)	13	4.73 ±0.39	≤1.32	15	$1.6 \times 10^3 \pm 421.5$	67 ±18	95.8
Scour and bleach (MQ–111 B)	10	4.58 ±0.28	≤1.32	11	$1.2 \times 10^3 \pm 380.7$	92 ±29	92.3

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Mean ± standard error.
[c]Unwashed values are from MQ–111 A.
[d]Percent change in viable bacteria or endotoxin.

Table 5.10—Evaluation of Microbiological Contaminants on Heavily Contaminated Cotton Washed on the Continuous Batt System (MQ–113)

Treatment[a]	GNB (log_{10}CFU/g)[b]		Endotoxin (ng/g)[b]		GPB (log_{10} CFU/g)[b]		Fungi (log_{10} CFU/g)[b]	
	Unwashed[c]	Dried	Unwashed[c]	Dried	Unwashed[c]	Dried	Unwashed[c]	Dried
Wash only (MQ–113 B)	5.61	2.31	5.5×10^4	750	ND	ND	ND	ND
Wash only (MQ–113 B2)	5.40	<1.40	9.3×10^4	890	6.59	3.28	3.60	<1.70
Wash and bleach (MQ–113 H)	5.59	<1.40	1.0×10^5	370	6.60	2.85	3.58	<1.70
Scour and bleach (MQ–113 I)	5.43	<1.40	9.3×10^4	180	6.41	2.78	3.88	<1.70

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Means of triplicate determinations.
[c]Unwashed values from MQ–113 A.
ND = No data.

treatments was shown to completely remove the acute biological reactivity from this contaminated cotton (chapter 4), and an additional trial was done to evaluate the possibility that the washed fiber was being recontaminated by the accumulation of endotoxin in the finish bath of the continuous batt system. During normal washing conditions the finish solution is recirculated with periodic input of fresh solution to replace that taken out by the fiber. However for this trial the finish solution was not recirculated; therefore the washed cotton was continuously exposed to fresh solution. The results of this trial (MQ–139) are given in table 5.11. The data show no accumulation of endotoxin on the lint or in the finish solution for any of the treatments. As with previous trials, the wash-and-bleach treatment reduced the levels of endotoxin more than the wash-only treatment. Therefore it appears that recirculation of the finish solution is not a source of recontamination of the washed fiber with endotoxin in this system; however, additional studies must be done to confirm these results.

Mechanical Cleaning of Cotton

Before washing at the Cotton Incorporated facility, lint was processed through a commercial device called the COTTONMASTER®. This device is designed as a fiber cleaner that cleans, separates, and parallels the fiber before the formation of a batt for wet processing. Use of this cleaning device reduced the levels of GNB up to 25 percent of the levels of unprocessed, unwashed fiber; however, no reduction was observed in the levels of endotoxin on the samples. These data suggest that although mechanical processing of cotton removes trash from the fiber, the bacteriological quality of the fiber remains unimproved (*23*).

Effect of Storage

Microbiological analysis of unwashed lint stored for 12 to 15 months showed essentially no change in the levels of GNB or endotoxin, thus suggesting the stability of the general microbiological characteristics of stored cotton (table 5.12). The same trend was observed in washed lint. Samples of washed lint stored for 12 to 15 months showed that both the endotoxin and the GNB remained constant or decreased at most by 1 log. These data indicate that the storage of washed cotton under conditions that generally prevail throughout the cotton industry will not expose the cotton to an environment that is conducive to the regrowth of bacteria. However, the genera or species/strains may differ and were not addressed by this study.

Table 5.11—Effect of Recirculation of Finish on Recontamination of Washed Fiber with Endotoxin (MQ–139)

	LPS[c]		
Treatment[a] and sample[b]	Prefinish lint (ng/g)	Postdryer lint (ng/g)	Water (ng/ml)
Wash only, recirculation (MQ–139 B)			
A–1	75	100	2.5
2	250	250	2.5
3	375	375	2.5
4	750	750	12.5
Wash only, no recirculation (MQ–139 C)			
B–1	750	750	0.1
2	500	750	2.5
3	1000	750	2.5
4	500	3000	2.5
Wash and bleach, no recirculation (MQ–139 D)			
C–1	150	225	0.3
2	175	150	2.5
3	375	100	1.0
4	200	150	1.3

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Unwashed lint MQ–139 A—1.3×10^5 ng/g; tapwater—0.1 ng/ml.
[c]Means of triplicate determinations.

Table 5.12—Effect of Storage on the Levels of GNB and Endotoxin on Washed Cotton

	Washing		Microbiological analysis			
			Original analysis		September 1982	
Treatment[a]	Temperature	Date	GNB[b]	Endotoxin[c]	GNB[b]	Endotoxin[c]
MQ–89 E	49 °C	6/8/81	5.23	400	2.78	420
MQ–89 F	49 °C	6/8/81	3.52	400	3.18	420
MQ–91 B	60 °C	9/10/81	2.18	400	1.32	420
MQ–91 C	60 °C	9/10/81	2.57	300	1.32	42
MQ–91 F	60 °C	9/22/81	1.90	40	1.32	42
MQ–89 G	Unwashed[d]	—	4.60	800	4.82	830

[a]Designations refer to cotton ID and washing treatment (see app. 2).
[b]Log_{10} CFU/g; means of duplicate or triplicate determinations.
[c]ng/g; means of triplicate determinations.
[d]Sample collected on 7/1/81.

Results of Airborne Analysis

Viable Microorganisms

In general, washing, bleaching, and scouring on the continuous batt system significantly reduced ($p = 0.05$) the amounts of microorganisms in airborne dust generated during carding of the treated cottons. Tables 5.13, 5.14, and 5.15 show results for bacteria from cottons MQ–107 and MQ–111, MQ–113, and MQ–139, respectively. These data show that a primary washing reduces the amounts of airborne bacteria in cotton dust. Additional treatments, e.g., bleaching and scouring, did not significantly reduce the levels of airborne bacteria below those achieved with the wash-only treatment. Furthermore, amounts of airborne bacteria in dusts from several cottons washed by modification of the primary washing process were comparable to each other.

As with the bacteria, airborne fungi were reduced by a primary washing treatment, and bleaching and scouring did not reduce further the amounts of fungi in cotton dust.

Studies of thermophilic actinomycetes (TA) represented by *Thermoactinomyces* spp., were not as extensive as those for other bacteria or for fungi. Only air samples of dust from unwashed MQ–139 cotton and the correspond-

Table 5.13—Effect of Washing, Bleaching, and Scouring on the Concentrations of Respirable Particles Bearing Bacteria and Fungi in Airborne Dust From Treatments MQ–107 and MQ–111

		Bacteria			
Treatment[a]	Dust level (mg/m^3)	*n*	Total (log_{10} CFU/m^3)[b]	Gram-negative (log_{10} CFU/m^3)[b]	Fungi (log_{10} CFU/m^3)[b]
Unwashed (MQ–111 A)	0.50	4	4.19 a	3.68 a	3.50 a
Unwashed (MQ–107 A)	0.25	8	4.14 a	2.81 b	2.76 b
Scour and bleach (MQ–111 B)	0.45	24	2.58 b	1.76 c	2.43 c
Wash and bleach (MQ–111 C)	0.35	8	2.45 b	1.64 c	2.48 c
Wash only (MQ–111 D)	0.30	16	2.43 b	1.68 c	2.44 c
Clean room	0.03	4	2.40 b	1.61 c	2.33 c

[a]Designations in parentheses refer to cotton ID and treatment (see app. 2).
[b]Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 5.14—Effect of Washing and Bleaching Cotton Treatment MQ–113a on Concentrations of Particles Bearing Bacteria in Airborne Dust

	Bacteria	
Treatment[a]	Total (log_{10} CFU/m^3)[b]	Gram-negative (log_{10} CFU/m^3)[b]
Standard (MQ–113a A)	4.89 a	3.27 a
Wash with Washaid 1173, Rolls out (MQ–113a B)	3.13 de	2.43 cd
Wash with Triton X–100, Rolls in (MQ–113a C)	3.36 cd	2.64 cd
Wash with Washaid 1173, Rolls in (MQ–113a D)	3.41 c	2.81 bc
Wash with Washaid 1173, Rolls out (MQ–113a E)	3.26 cde	2.59 cd
Wash and bleach with Washaid 1173, rolls out (MQ–113a F)	3.06 e	2.29 d
Wash and bleach with Washaid 1173, rolls in (MQ–113a G)	3.79 b	3.19 ab
Clean room (MQ–113a CR)	2.30 f	1.56 e

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Values in the same column followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 5.15—Concentrations of Respirable Particles Bearing Bacteria or Fungi in Airborne Dust From Washed and Unwashed Cotton (MQ–139)

Treatment[a]	Bacteria		Fungi (log_{10} CFU/m^3)[b]	*Thermoactinomyces* (log_{10} CFU/m^3)[b,c]
	Total (log_{10} CFU/m^3)[b]	Gram-negative (log_{10} CFU/m^3)[b]		
Unwashed (MQ–139 A)	2.99 a	1.87 a	2.61 a	3.69 ± 0.15
Wash only (not recirculated) (MQ–139 C)	2.69 bc	1.45 b	2.19 bc	—
Wash only (recirculated) (MQ–139 B)	2.53 c	1.57 ab	1.99 c	—
Wash and bleach (not recirculated) (MQ–139 D)	2.74 b	1.65 ab	2.11 c	—
Clean room (MQ–139 CR)	2.51 c	0.77 c	2.34 b	1.90 ± 0.06

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.
[c]± Standard deviation.

ing clean room were assayed. Dust from unwashed MQ–139 cotton contained approximately 4800 CFU/m^3 whereas the clean room had 80 CFU/m^3. No air samples were obtained of dust from the corresponding washed cottons from MQ–139. However, analyses of the washed bulk fibers show that washing reduced the amounts of TA on cotton fiber (table 5.16) for cottons MQ–113 and MQ–139. Although analysis of fiber cannot substitute for a complete characterization of the microbial content in the dust, it does indicate a relative reduction in TA for the washed samples. Dust from fiber samples that had low TA would be expected to have low amounts of TA also, because there is generally good agreement between the microbial content of fiber and that of the airborne dust (table 5.17).

The highest degree of association between bacterial contents of dust and fiber was obtained for total bacteria (table 5.17). Gram-negative bacterial content of airborne dust was not consistently associated (R^2, $p < 0.05$) with the

Table 5.16—Amounts of *Thermoactinomyces* spp. on Cotton Fibers Prior to Carding

Treatment[a]	*Thermoactinomyces* (log_{10} CFU/g)[b]
Unwashed MQ–107 A	2.09
Wash and bleach MQ–107 B	0
Unwashed MQ–111 A	0
Scour and bleach MQ–111 B	0
Wash and bleach MQ–111 C	1.77
Wash only MQ–111 D	0
Unwashed MQ–113 A	7.73
Wash only MQ–113 B	6.41
Wash and bleach MQ–113 H	3.26
Unwashed MQ–139 A	5.00
Wash only, finish recirculated MQ–139 B	3.45
Wash only, finish not recirculated MQ–139 C	3.40
Wash and bleach MQ–139 D	1.18

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Values are from duplicate determinations.

Table 5.17—Regression Relationship and Correlation Between the Bacterial Content of the Cotton Fiber Prior to Carding and the Amounts of Airborne Particles Bearing Bacteria in Card-Generated Dust (MQ–107, –111, –113a, and –139)

Cotton ID	Bacteria	Bacteria on cotton fiber[a]		Regression equation[b] (log_{10} PBB/m^3)	n[c]	R^2
		Unwashed	Washed			
MQ–107, MQ–111	Total	4.79	2.01	1.31 + 0.58 Lap	5	0.98*
		5.75	2.82	0.92 + 0.55 Bale	5	0.99*
	GNB	3.54	1.90	0.60 + 0.61 Lap	5	0.71 NS
		4.52	2.03	0.64 + 0.50 Bale	5	0.95*
MQ–113a	Total	6.79	3.14	1.66 + 0.48 Bale	9	0.92***
		7.10	3.06	1.45 + 0.49 Bale	13	0.94***
	GNB	5.14	2.62	1.31 + 0.39 Bale	9	0.77**
		5.16	2.63	1.71 + 0.26 Bale	13	0.79***
MQ–139	Total	5.41	3.05	2.28 + 0.13 Lap	35	0.29**
	GNB	5.31	1.90	1.42 + 0.08 Lap	35	0.09 NS
	Thermoactinomyces	5.20	2.85	5.73 + 0.39 Lap	3	0.75 NS

[a]Values are the means in log_{10} MPN/g for the bacterial group (Total or GNB) and the fiber sample (lap or bale).
[b]The general form of the equation is $Y = b_0 + b_1 X$; $Y = log_{10}$ (PBB/m^3); PBB = particles bearing bacteria; Lap = log_{10} (MPN/g of lap fiber); Bale = log_{10} (MPN/g of bale fiber); b_0 = y intercept; b_1 = slope.
[c]N = number of observation pairs.
***$p \leq 0.0001$; **$p \leq 0.001$; *$p \leq 0.01$; NS = not significant.

bacterial content of the fiber. The heterogeneity of bacteria in fiber samples and the influence of carding rate and dust level on the bacterial content of airborne dust (*24*) may account for some of the lack of association.

Analysis of the size distribution of the airborne particles bearing microorganisms (table 5.18) shows that after washing (or modifications of the primary washing process), bleaching, and scouring, the median aerodynamic diameter of particles bearing bacteria is increased relative to particles bearing bacteria from unwashed cotton or the clean room. Size distributions of airborne particles bearing fungi are affected less by the washing, bleaching, or scouring treatments than are particles bearing the bacteria. The median aerodynamic diameter of particles bearing TA in unwashed MQ–139 dust was 2.48 ± 1.92 μm.

Endotoxin Levels

In every trial exposure, washing the bulk cottons before carding resulted in substantial reductions in the endotoxin contents of the lint and of the airborne dusts. Consequently, the concentration of endotoxins per unit volume of air was reduced similarly. Table 5.19 (MQ–79) shows that dusts from cottons that were grown in three distinct growing regions—California, Mississippi, and Texas—were each affected markedly by prior washing of the baled cottons. On a dust weight basis, endotoxins in dusts collected on personal sampler filters were reduced by 64.5 percent, 95.6 percent, and 94.5 percent for California, Mississippi, and Texas cotton dusts, respectively. The concentrations of endotoxins in the air during the human exposure trials were reduced by prior washing of the cottons by 69.2 percent, 95.7 percent, and 94.7 percent for the three cottons. These changes in airborne concentrations were not due to alterations in the airborne dust levels, because each exposure was to approximately 0.5 mg/m^3 of vertically elutriated dust (*25*). Of interest, dusts from the unwashed cottons, showed very different levels of endotoxin contamination, with the dust from Texas as the most contaminated and the dust from California as the least contaminated. Studies of the effect of area of growth, classers' grade, and variety of cotton on subsequent contamination of carded dusts with gram-negative bacterial endotoxins are presented elsewhere (*26*).

Mississippi cottons were used additionally in a study (MQ–79) of the effects of altering the washing conditions on the removal of endotoxins and ultimately on the acute pulmonary function response of the human subjects to the dusts. Washing procedure (rayon rinse and wool scouring) was varied, as were the water temperature (28 °C and 66 °C) and the water-to-fiber ratio. Table 5.20 shows again that washing of the Mississippi cottons before carding effectively removes the endotoxins from the carded dusts. The efficacy of removal varied slightly among the different condi-

Table 5.18—Median Size Distributions of Respirable Particles Bearing Bacteria and Fungi in Airborne Dust From Washed and Unwashed Cotton Treatments MQ–107, –111, –113a, and –139

Treatment[a]	*n*	Bacteria		Fungi (μm)[b]
		Total (μm)[b]	Gram-negative (μm)[b]	
Unwashed (MQ–107 A, MQ–111 A)	12	3.33 ± 1.65	3.24 ± 1.61	4.45 ± 1.51
Scour and bleach (MQ–111 B)	24	6.32 ± 1.83	6.57 ± 1.92	4.47 ± 1.52
Wash and bleach (MQ–111 C)	8	6.28 ± 1.81	7.04 ± 1.72	4.87 ± 1.42
Wash only (MQ–111 D)	16	6.53 ± 1.81	7.31 ± 1.77	5.00 ± 1.59
Clean room	4	5.29 ± 1.77	5.57 ± 1.71	4.91 ± 1.42
Unwashed (MQ–113a A)	12	2.77 ± 1.62	4.34 ± 1.57	—
Wash, WARO (MQ–113a B)	8	4.87 ± 1.87	5.68 ± 1.54	—
Wash, TXRI (MQ–113a C)	2	4.16 ± 1.83	5.27 ± 1.52	—
Wash, WARI (MQ–113a D)	2	4.59 ± 1.82	5.37 ± 1.57	—
Wash, WARO (MQ–113a E)	2	3.90 ± 1.81	5.00 ± 1.59	—
Wash and bleach, WARO (MQ–113a F)	2	4.15 ± 1.89	5.97 ± 1.65	—
Wash and bleach, WARI (MQ–113a G)	2	3.50 ± 1.79	3.81 ± 1.58	—
Clean room	4	5.27 ± 1.89	4.73 ± 1.64	3.78 ± 1.41
Unwashed (MQ–139 A)	12	2.75 ± 1.81	4.34 ± 1.52	3.61 ± 1.40
Wash only (MQ–139 B)	8	3.78 ± 1.85	5.62 ± 1.74	3.93 ± 1.43
Wash only (MQ–139 C)	8	4.38 ± 1.83	6.36 ± 1.61	4.64 ± 1.45
Wash and bleach (MQ–139 D)	8	4.26 ± 1.71	5.67 ± 2.00	4.40 ± 1.43
Clean room	8	2.79 ± 1.83	7.79 ± 1.84	3.45 ± 2.40

[a]For details of the treatment/processing, see app. 2. Abbreviations refer to Washaid 1173 (WA), rolls out (RO), Triton X–100 (TX), rolls in (RI).
[b]Values are the mean ± standard error of the median aerodynamic diameters (μm) of particles.

tions used. As measured by the personal sampler filters, washing on the rayon rinse system with the higher water temperature (66 °C) and lower water-to-fiber ratio (50:1) was the most efficient in removing endotoxins from the dust (95.6 percent) and therefore reducing the airborne concentration (95.7 percent). The other techniques, however, were still highly efficient with a range of 83.0–89.3 percent removal of endotoxins on a dust weight basis when compared to the levels when unwashed cotton was carded. As in the previous trials, the gravimetric dust levels were kept relatively constant (0.4–0.6 mg/m^3) during this washing study (25).

Table 5.19—Effect of Washing on Endotoxin Content of Airborne Dusts From Cottons Grown in California, Mississippi, and Texas (MQ–79)

Area of growth and treatment[a]	*n*	Endotoxin[b]	
		Dust (ng/mg)	Air (ng/m^3)
California, unwashed (MQ–79 B)	17	40.3 ± 4.1	23.7 ± 1.9
California, wash (MQ–79 C)	16	14.3 ± 2.2	7.3 ± 1.0
Mississippi, unwashed (MQ–79 F)	16	167.6 ± 7.6	87.6 ± 4.5
Mississippi, wash (MQ–79 G)	16	7.4 ± 1.0	3.8 ± 0.5
Texas, unwashed (MQ–79 D)	17	390.2 ± 22.6	202.2 ± 13.2
Texas, wash (MQ–79 E)	16	21.6 ± 4.0	10.8 ± 2.1

[a]Designations in parentheses refer to cotton ID and treatment (see app. 2).
[b]Mean ± standard error.

Table 5.20—Effect of Washing Conditions on Endotoxin Content of Airborne Cotton Dusts (MQ–79)

Treatment[a]	Endotoxin[b] Dust (ng/mg)	Endotoxin[b] Air (ng/m^3)
Unwashed (MQ–79 F)	167.6 ± 7.6	87.6 ± 4.5
Rayon rinse system, 28 °C, 65:1 (MQ–79 I)	28.5 ± 1.6	12.5 ± 0.8
Rayon rinse system, 66 °C, 65:1 (MQ–79 J)	17.9 ± 2.0	10.6 ± 1.1
Rayon rinse system, 66 °C, 50:1 (MQ–79 G)	7.4 ± 1.0	3.8 ± 0.5
Wool scouring system, 66 °C (MQ–79 H)	22.0 ± 1.8	12.9 ± 1.1

[a]Mississippi cotton, washing system, water temperature (°C), and water-to-fiber ratio. Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Mean ± standard error.

Vertically elutriated cotton dusts from MQ–107 and MQ–111 cottons represent samples from a study that varied the washing treatments of the baled cottons before carding. Table 5.21 shows the endotoxin contents of the airborne dusts and the resulting concentrations per unit of air when cottons that were unwashed, washed only, or washed and treated with bleach or bleach and caustic scour were carded. All treatments were equally effective in reducing the quantity of endotoxins in the vertically elutriated carded cotton dusts (95.3 percent, wash and bleach; 96.2 percent, wash only; 98.0 percent, scour and bleach). The resulting changes in airborne concentrations were reduced by 96.4 percent–97.9 percent compared with the unwashed cottons.

Table 5.22 shows data obtained during the exposure trials for cottons designated MQ–113. The efficacy of washing cotton that was initially of high microbiological content was tested (unwashed and wash-only rows of codes A and B of table 5.22). Cotton that was contaminated with high levels of microbial flora was washed, and the endotoxin content of the airborne dust was effectively reduced (94.4 percent) when compared with the vertically elutriated dusts of the unwashed cotton. A 91.0 percent reduction in endotoxin concentration per unit volume of air was obtained.

A minor additional study with MQ–113 cottons included varying washing techniques and treatments. When vertically elutriated dusts from the carding of cottons that were washed or treated differently from code B (table 5.22) were analyzed for endotoxin content, each method was highly efficient at reducing the endotoxin contamination of the dusts (89.9–97.2 percent) when compared to the endotoxin level in the dust from unwashed cotton.

Table 5.21—Effect of Washing Treatments on Endotoxin Content of Airborne Cotton Dusts From Standard Cotton (MQ–107, MQ–111)

Treatment[a]	n[b]	Endotoxin[c] Dust (ng/mg)	Endotoxin[c] Air (ng/m^3)
Unwashed, standard cotton (MQ–107 A) (MQ–111 A)	25	133.15 ± 10.06	62.55 ± 7.39
Wash and bleach (MQ–107 B) (MQ–107 B2) (MQ–111 C)	16	6.30 ± 0.88	2.24 ± 0.22
Wash only (MQ–111 D)	13	5.04 ± 0.38	1.71 ± 0.17
Scour and bleach (MQ–111 B)	6	2.63 ± 0.45	1.31 ± 0.18
Clean room (CR)	12	—	ND

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Each n = 2–4 VE filter extracts combined and endotoxins performed in duplicate.
[c]Mean ± standard error.
ND = Not detectable.

Table 5.22—Effect of Washing High Microbiological Content Cotton on Endotoxin Content of Airborne Carded-Cotton Dust (MQ–113a)

Treatment[a]	n[b]	Endotoxin[c] Dust (ng/mg)	Endotoxin[c] Air (ng/m^3)
Unwashed (MQ–113a A)	14	201.83 ± 18.86	50.87 ± 6.09
Wash only, Washaid, 1173 rolls out (MQ–113a B)	24	11.41 ± 2.17	4.56 ± 0.76
Wash only, Triton–X 100, rolls in (MQ–113a C)	2	6.92 ± 0.98	3.67 ± 0.52
Wash only, Washaid 1173, rolls in (MQ–113a D)	2	20.46 ± 1.69	8.60 ± 0.41
Wash only, Washaid 1173, rolls out (MQ–113a E)	2	8.42 ± 0.13	4.04 ± 0.23
Wash and bleach, Washaid 1173, rolls out (MQ–113a F)	2	5.57 ± 0.16	2.84 ± 0.03
Wash and bleach, Washaid 1173, rolls in (MQ–113a G)	2	20.38 ± 0.44	9.40 ± 1.02
Clean room (CR)	4[d]	—	0.48 ± 0.09

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Each n = 2–4 VE filter extracts combined and endotoxins performed in duplicate.
[c]Mean ± standard error.
[d]Represents 2 clean room days. A 3d day provided filters on which no endotoxins were detectable.

Washing treatments were studied again during the exposure trials of MQ–139 cotton. Table 5.23 shows the results of endotoxin analyses of vertically elutriated dusts collected during the carding on unwashed, washed, and washed-and-bleached cottons. A variable in the washing procedure was recirculating or not recirculating the finish bath water. On a dust weight concentration basis, the mean endotoxin contents for dusts from wash-only-recirculated and wash-only-not recirculated were nearly identical. Both methods of washing the cotton resulted in the reduction of endotoxins in the airborne dusts of 97.9 percent compared with that of unwashed cotton. The addition of the bleaching procedure resulted in slightly higher mean endotoxin contamination of the airborne dust (44.74 ng/mg). However, the effective reduction of endotoxin content in the carded cotton dust was still 95.8 percent compared with that of unwashed cotton. Airborne levels of endotoxins (ng/m^3) were reduced by all treatments by 91–97 percent compared with that of unwashed cotton.

Vertical elutriators were operated in the exposure rooms when only clean air was used as the exposure. Extraction of the filters from those units and analyses for endotoxin levels in the air consistently resulted in mean levels which were either not detectable by the procedure used or less than 0.6 ng/m^3. Airborne endotoxin levels on the days designated "clean room" were therefore negligible.

Table 5.23—Effect of Washing Conditions on Endotoxin Content of Airborne Cotton Dusts (MQ–139)

Treatment[a]	n[b]	Endotoxin[c] Dust (ng/mg)	Endotoxin[c] Air (ng/m^3)
Unwashed (MQ–139 A)	13	1074.02 ± 140.40	142.70 ± 19.81
Wash only, finish recirculated (MQ–139 B)	16	22.64 ± 3.55	4.89 ± 1.00
Wash only, finish not recirculated (MQ–139 C)	16	22.92 ± 4.14	5.95 ± 0.97
Wash and bleach, finish not recirculated (MQ–139 D)	14	44.74 ± 12.40	12.55 ± 3.82
Clean room (CR)	8	—	0.56 ± 0.12

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Each n = 2–4 VE filter extracts combined and endotoxins performed in duplicate.
[c]Mean ± standard error.

Discussion

Analysis for Bacteria and Endotoxin on Fiber

For all systems tested, washing substantially reduced the levels of both GNB and endotoxin on the washed fiber. In general, GNB counts and endotoxin were reduced by more than 90 percent from unwashed controls, and as more experience was gained by using different systems and different treatments, reductions of 99 percent or greater were achieved for some trials. The reductions in contamination were due to both the washing treatments and the high temperatures necessary for drying the fiber. Washing treatments include the particular treatment such as bleaching, the temperature of the system, the water-to-fiber ratio, and any mechanical agitation associated with a particular washing system. All of these factors contributed to the removal of GNB and endotoxin from the fiber: however, from the results of the fiber analysis it was impossible to distinguish any one factor as contributing more than another to the removal of either GNB or endotoxin. In general, the more rigorous the treatment, the greater the reduction in contamination on the fiber.

Fiber with different levels of contamination was evaluated on the continuous batt system and the rayon rinse system. In both systems a greater percentage of GNB was removed from the fiber when washing the more contaminated cotton; but the absolute levels of contamination remaining on the washed fiber were greater than the levels remaining on the washed fiber with less initial contamination. Therefore, an awareness of the degree of initial contamination of an unwashed cotton will be important in determining the conditions and possibly the system for washing if the objective is to remove microbiological contaminants.

Evaluations were also done for total bacteria, gram-positive bacteria, and fungi on several samples of washed lint. For all washing treatments tested, the total bacteria, gram-positive bacteria, and fungi paralleled the patterns observed for the GNB. These data show that the washing procedures are not selective for a particular contaminant and that measurement of one contaminant reflects the relative change in levels of other microbiological contaminants associated with the fiber.

Samples of washed cottons were stored and evaluated 1 year later to determine if there were changes over time in the levels of GNB and endotoxin. In general, cotton is stored in a warehouse that provides protection from direct exposure to the environment but does not control daily changes in temperature and humidity. The bale consists of approximately 227 kg of lint packed to a density of 0.78 kg/m^3 and wrapped in polypropylene or hemp bagging. On storage the fiber generally contains from 5 percent to 10 percent moisture on a dry weight basis; therefore unless the bale is stored where it can get wet, the conditions of storage are fairly constant. For all samples the level of both GNB and endotoxin either dropped or did not change over the time of storage. These results probably reflect the constant environment normally found in cotton warehouses as well as the reduction of materials on the washed fiber that can be used as a substrate for biological growth.

Analysis for Airborne Viable Microorganisms

Washing was generally as good as the more involved treatments, such as scouring or bleaching, in terms of reducing bacteria and fungi in airborne dust. Thermophilic actinomycetes may be more thoroughly reduced by supplementing washing with bleaching, however, more studies must be done before definite conclusions can be drawn, because only a limited number of analyses was made. None of the washing, bleaching, or scouring treatments resulted in an increase in microbes on the treated fibers or the dust generated from carding the fibers. The amounts reported here for gram-negative bacteria in dust from three of four unwashed and washed cottons are of the same magnitude as those reported by Bergstrom et al. (*27*) in their studies on the effects of washed cotton. No comparable data are available for fungi or TA. Intense exposure to TA may cause respiratory distress in sensitized individuals; however, airborne levels of TA in the tests reported here were far below those associated with the mouldy hay tests (*28*). Furthermore it has been shown that dust from cotton with virtually no TA (*24*) elicited a strong acute pulmonary response in individuals inhaling the dust (*29*).

The median (aerodynamic) sizes of airborne bacteria and TA are consistent with the bronchial rather than alveolar nature of the human response to cotton dust. Washing, bleaching, and scouring generally have comparable effects on the size distribution of particle bearing microbes in dust and result in increased median sizes for airborne particles bearing bacteria. However, these processes have little effect on the size distribution of particles bearing fungi in airborne dust.

The association between amounts of airborne bacteria and the bacterial content of lap and bale fiber is strongest for total bacteria. The association is also very strong in some treatments for gram-negative bacteria. The lack of association between these parameters for some treatments is probably due to the very heterogeneous distribution of gram-negative bacteria on cotton fiber. Such distributions make representative sampling difficult and indicate the need for high numbers of replicate determinations for microbiological parameters on fiber. In contrast, the microbiological content of airborne dust represents a sample of many fibers. In essence, the blending and the carding process provided a mixing mechanism.

Analysis for Airborne Endotoxins

Prior washing of raw cottons effectively reduces the endotoxin content on the lint and in the airborne dust generated during carding. Cottons that were grown in different geographic areas or that were chosen purposefully for their high levels of microbial contamination similarly showed marked decreases in the amounts of endotoxins per unit of air during carding when compared to the concentrations of airborne endotoxins during the carding of their unwashed counterparts. At similar gravimetric dust levels (*25*), markedly decreased airborne concentrations of endotoxins were observed in dusts from carded washed cottons, indicating that the changes in the airborne endotoxins were of considerable proportion. The data were presented in terms of levels both in the dust and in the air in order to demonstrate that significant changes in the airborne concentrations of endotoxins could be achieved by washing the cottons before carding. Perhaps, however, airborne concentrations in nanograms per cubic meter may not be the most appropriate values to use when comparison of different washing techniques or conditions is the goal or when the effects of different varieties or growing areas of cottons are to be considered. Endotoxin levels that are based on the vertically elutriated or personal sampler gravimetric dust weight basis may be more appropriate values to compare.

Endotoxin levels in the airborne dusts can be quantified in cotton dusts collected on personal sampler filters that were hung on the subjects (tables 5.19 and 5.20) or from properly collected vertical elutriators that sampled the air at various areas of the exposure rooms (tables 5.21–5.23). All aerodynamically sized fractions of airborne carded cotton dusts contain quantifiable endotoxins (*25*). In addition, results from separate experiments confirmed that replicate carding of the same cotton 1–2 months after the first trial produced strikingly consistent endotoxin contamination in the airborne dusts (*26*). Therefore, for multiple carding trials with the same cotton, the data were combined.

Examination of the endotoxin levels in the dust from unwashed carded cottons in the MQ–79 study (table 5.19) showed that the endotoxin contamination of the dust can vary markedly for cottons grown in different geographic locations. Dusts from carded unwashed California cotton contained a mean endotoxin concentration of 40.3 ng/mg, which was approximately fourfold less than in dusts from Mississippi cotton and over ninefold less than in dusts from Texas cotton. This difference in endotoxin contamination of carded cotton dusts was observed additionally in a separate area of growth study in which Texas cottons generated the least amounts of endotoxins in the carded cotton dusts, while the dust from carded Mississippi cottons contained the heaviest contamination (*26*). The data from these two studies, in conjunction with those from a third study in which baled cottons from worldwide sources were tested for endotoxin contamination (*30*), reinforce the observation that area of growth may affect the endotoxin contamination of the cottons and their respective airborne dusts. Likewise, dusts from cottons of the same classers' grade, but which were grown in different geographic areas, showed marked differences in their endotoxin contents (*31*). Although the washed cotton studies do not address these differences specifically, it was suggested that differences in endotoxin content of the airborne dusts may be related in part to the variety of cotton plant (*26*); however, confirmation of those data should be pursued. The greatest impact on the endotoxin contamination of the cottons and their dusts appears to be from the local overall growth condition in the different geographical areas.

Variations in the washing treatments can result in differences in the concentration of endotoxins in the carded cotton dusts compared with dusts from unwashed cottons. These differences among the various regimens are small, however, in light of the marked differences noted in dusts from unwashed and washed carded cotton. The data from table 5.20 show that hot water (66 °C) washing at water-to-fiber ratio of 50:1 was most efficient in removing endotoxins. Although hot water at the higher ratio of 65:1 was less efficient numerically, the real biological signficance of this apparent discrepancy is unknown. Similarly, the addition of bleach or a caustic and bleach (tabies 5.21–5.23) produced little difference in the endotoxin contamination of the vertically elutriated dust compared with the effective removal of endotoxins by washing alone. Mean endotoxin concentrations of similar magnitude resulted for both recirculating and not recirculating the finish bath water (table 5.23). It must be recognized, of course, that the study of different washing treatments has importance and utility beyond any isolated effect on endotoxins. It should be noted additionally that perhaps the most effective washing treatment for reducing endotoxins from cottons has yet to be tested.

In conclusion, water washing of raw cottons before carding is an effective method for reducing the endotoxin content in the lint and subsequently card-generated airborne dust, regardless of the source or condition of the cotton. Variations in the washing treatments can affect the efficacy of endotoxin removal. Endotoxin analyses can provide a simple, reproducible, and reliable method for monitoring the "cleanliness" of the airborne cotton dust. Acute human pulmonary reactivity as measured by the forced expiratory volume in 1 second (FEV_1) after exposure to cotton dusts is reported in chapter 4 and has been shown to correlate well with the endotoxin content of the vertically elutriated dusts (*26, 29, 31*). The data therefore suggest that quantification of airborne endotoxins may reflect the "potency" or "toxicity" of the carded cottons or their dusts as well. Although these studies do not necessarily imply that gram-negative bacterial endotoxins are the causative agent(s) of the acute or chronic pathophysiology induced by cotton dust inhalation, they do

suggest that quantification of endotoxins in the card-generated cotton dusts provides a biological indicator of pulmonary reactivity.

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Chapter 6
Differentiation of Washed and Unwashed Cotton

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Figure:

Introduction

An adequate definition or objective criteria for characterizing washed cotton is necessary for compliance with any standards covering washed cotton and for distinguishing washed from unwashed cotton. The cooperative studies at Clemson, South Carolina, provided a unique opportunity to study a battery of analyses that could be correlated with panel response data. As it would be impractical to use human response to characterize all washed cotton, objective measurements that correlated with panel response data were the preferred goal. Therefore, research was undertaken to define washed cotton using objective criteria correlated to sample history and lung function data from the cooperative studies at Clemson.

Research data collected and analyzed at USDA, Agricultural Research Service, Southern Regional Research Center proved useful in deciding the analytical tests appropriate for preliminary study; these data were concerned with the chemical composition of the cotton fiber (1) as well as the early attempts at washing cotton (2). The most useful source of information came from the proximate and elemental analyses already performed at the Southern Regional Research Center on cottons collected throughout the country as well as the more recent cottons run in the cooperative Clemson studies (3–7). After several tests were chosen for initial experimentation, data were collected at several different laboratories and statistically analyzed to determine those analyses that would be used in more extensive studies.

Analyses Investigated

Some initial characterization of washed cotton (*8*) and work by Muller et al. (*9*) indicated that a combination of nitrogen contents and boiling water extractables could be used to predict the effectiveness of washing treatments. Data in table 6.1 show that nitrogen contents of the washed with finish[3] (W/F) and the washed (W) cottons have been reduced compared with the unwashed samples (UW). Wide variations in the cottons grown in the three areas illustrate why nitrogen values alone should not be used as a marker for washed cotton. However, by combining boiling water extractables (BWE) with nitrogen analyses, the quality of the wash can be determined.

Although this served as a beginning, more research was needed to determine a simple and cost-effective analysis for differentiating washed cotton regardless of the growing location.

Comparative data on boiling water extractables (BWE), ash contents (ASH), water soluble reducing substances (WSRS), and conductance (CON) were reported by Berni et al. (*10*).

Boiling water extraction (BWE) was effected by ovendrying 1 g replicates of a cotton sample which were immersed in 75 ml of boiling deionized water for 30 minutes and centrifuged at 1850 rpm for 5 minutes to remove excess liquid. The extraction and centrifugation steps were repeated with 5 ml of water, and the cotton was then ovendried to constant weight in tared weighing bottles. BWE was calculated using weight loss of a sample.

Ash contents (ASH) were determined gravimetrically on cottons incinerated at 750 °C and were reported as percent of original sample weight.

Water soluble reducing substances (WSRS) were determined by comparing the reducing ability of the cold water surfactant extract of the cotton to that of a standard reducing substance, glucose, by use of a method described by Perkins (*11*). The method consists of reaction of an aliquot of the water extract with an excess of alkaline potassium ferricyanide and back titration with standard ceric sulfate in acid solution in the presence of o-phenathroline ferrous sulfate indicator. Values are reported as percent sugar content.

Conductance (CON) was measured with a digital a.c. conductivity bridge with platinized electrodes (*12*). Samples (200 mg) of Wiley milled fiber were mixed with 20 ml of deionized water, then shaken periodically for 1 hour. Suspended matter was removed by filtration, and meter readings were immediately obtained. Values are reported as micromhos.

A description of the 17 test cottons appears in table 6.2. For a test, four replicates of each cotton were made by randomly pulling and combining tufts from the quantity of

[3]SSC Finish 641, a nonionic finishing agent from SSC Industries, was used in order to facilitate processing.

Table 6.1—Nitrogen Content of Cottons From the 1981 Acute Exposure Level Studies on Washed Cotton[a] Before and After Boiling Water Extraction

Sample[c]	Nitrogen (%)[b]	
	Before	After
MQ–79 A, Mississippi UW	0.142 ± 0.013	0.121 ± 0.026
MQ–79 G, Mississippi W/F	0.120 ± 0.010	0.106 ± 0.011
MQ–79 GG, Mississippi W	0.107 ± 0.004	0.106 ± 0.007
MQ–79 B, California UW	0.290 ± 0.010	0.180 ± 0.010
MQ–79 C, California W/F	0.195 ± 0.004	0.181 ± 0.029
MQ–79 CC, California W	0.184 ± 0.005	0.174 ± 0.018
MQ–79 D, Texas UW	0.196 ± 0.005	0.136 ± 0.013
MQ–79 E, Texas W/F	0.145 ± 0.005	0.127 ± 0.010
MQ–79 EE, Texas W	0.131 ± 0.002	0.122 ± 0.009
Medical-grade cotton	0.008 ± 0.001	0.019 ± 0.010

[a]Cooperative studies performed at USDA's research facility, Clemson, South Carolina.
[b]Values represent the means ± standard deviations of percent nitrogen from 4 determinations.
[c]UW = unwashed; W/F = washed with finish to improve processing; W = washed without finish. See table 2 for further description of cottons and treatments.

Table 6.2—Description[a] of Cotton Samples

MQ number	Sample
79–1A DPL–55	Machine-picked cotton (1979 crop) used in exposures, unwashed cotton
79–1D DPL–55	Machine-picked cotton (1979 crop) washed cotton, rayon rinse system, Enka, 66 °C, 56:1 H_2O ratio, with finish
MQ–79 A	Mississippi screening (1981), unwashed control used in exposures
MQ–79 B	California standard, Acala SJ–5, unwashed control
MQ–79 C	California washed, rayon rinse system, Enka, 66 °C, 50:1 H_2O ratio, with finish (Acala SJ–5)
MQ–79 CC	California washed, rayon rinse system, Enka, 66 °C, 50:1 H_2O ratio, without finish (Acala SJ–5)
MQ–79 D	Texas standard, GSA–71 variety, unwashed control
MQ–79 E	Texas washed, GSA–71 variety, rayon rinse system, Enka, 66 °C, 50:1 H_2O ratio, with finish
MQ–79 EE	Texas washed, GSA–71 variety, rayon rinse system, 66 °C, 50:1 H_2O ratio, Enka, without finish
MQ–79 G	Mississippi DPL–61 washed, rayon rinse system, Enka, 66 °C, 50:1 H_2O ratio, with finish
MQ–79 GG	Mississippi DPL–61 washed, rayon rinse system, Enka, 66 °C, 50:1 H_2O ratio, without finish
MQ–79 H	Mississippi DPL–61 scoured, wool scouring system, 66 °C, with finish
MQ–79 HH	Mississippi DPL–61, wool scouring system, 66 °C, without finish
MQ–79–I	Mississippi DPL–61 washed, high volume, low temperature, rayon rinse system, Enka, 28 °C, 65:1 H_2O ratio, with finish
MQ–79–II	Mississippi DPL–61 washed, high volume, low temperature, rayon rinse system, Enka, 28 °C, 65:1 H_2O ratio, without finish
MQ–79–J	Mississippi DPL–61 washed, high volume, high temperature, rayon rinse system, Enka, 66 °C, 65:1 H_2O ratio, with finish
MQ–79–JJ	Mississippi DPL–61 washed, high volume, high temperature, rayon rinse system, Enka, 66 °C, 65:1 H_2O ratio, without finish

[a]See appendixes 2 and 3 for further description.
Source: Berni et al. (*10*).

cotton available at the Southern Regional Research Center. The resulting 68 grab samples were randomly ordered and numbered. A single determination of each chosen test was made on each sample. The identity and wash status of the cottons were unknown to those making the test measurements in order to obtain unbiased estimates of the errors associated with these determinations.

The means from the four replicates of each test were analyzed using discriminant analysis, a multivariate statistical technique used to classify observations into two or more groups on the basis of one or more variables (*13*). In addition, the data were subjected to multiple linear regressions to determine the nature of the relationships among ASH, CON, WSRS, and BWE. The ΔFEV_1 (change in forced expiratory volume at 1 second) values measured on subjects exposed to dust from these same cottons were used as dependent variables in regression models with values from the four analytical tests as independent variables.

Means and standard errors for each of the 17 cottons appear in table 6.3. Based on the within-sample variation, conductance showed the lowest coefficient of variation and ash, the highest. The coefficients were: conductance—7.1, boiling water extractables—23.5, water soluble reducing substances—42.0, and ash—56.6.

Single variate discriminant analyses (assuming equal group variances) of data in table 6.3 accurately classified washed and unwashed cotton. The discriminant analysis with CON data (table 6.4) accurately classified the washed status of all 17 cottons. Likewise ASH data (table 6.5) accurately classified 100 percent of the cotton samples. The discriminant analysis with WSRS (table 6.6) accurately classified all but one cotton (MQ–79 A). The BWE model also misclassified the same cotton (table 6.7).

The critical classification values used to determine the wash condition of the cottons are shown in table 6.8, and observed values from each laboratory test were independently compared with the critical values. A cotton was classified as washed if the observed value was less than the critical value.

It should be noted that the accuracy of these discriminant models in classifying these 17 cottons is an upper limit on how well the model can be expected to perform on

Table 6.3—Descriptive Statistics

Cotton and treatment[a]	Washed	Reducing substances[b]		Conductance[c]		Boiling water extractables[d]		Ash[e]	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
79–1A	No	0.3050	0.0194	17.125	0.2496	2.5926	0.2048	0.6700	0.1058
79–1D	Yes	0.0475	0.0048	5.450	0.2723	1.4650	0.0614	0.2225	0.0719
MQ–79 A	No	0.1375	0.0111	15.475	0.3987	1.6225	0.3550	0.6560	0.0240
MQ–79 B	No	0.3575	0.0048	27.550	0.6801	3.6350	0.0671	1.0025	0.2320
MQ–79 C	Yes	0.0350	0.0065	4.450	0.1708	1.1750	0.1326	0.3100	0.0236
MQ–79 CC	Yes	0.0275	0.0063	4.250	0.1190	0.7725	0.1687	0.2275	0.0888
MQ–79 D	No	0.2550	0.0685	23.450	0.1756	3.2400	0.1430	1.3400	0.2656
MQ–79 E	Yes	0.0450	0.0050	3.900	0.1472	0.8550	0.1079	0.2425	0.0361
MQ–79 EE	Yes	0.0400	0.0071	4.175	0.3010	0.7625	0.0998	0.2500	0.0481
MQ–79 G	Yes	0.0350	0.0029	4.500	0.4491	1.0325	0.1413	0.1975	0.0489
MQ–79 GG	Yes	0.0250	0.0029	3.925	0.2720	0.6050	0.1002	0.1550	0.0524
MQ–79 H	Yes	0.0300	0.0071	3.975	0.1377	0.8775	0.1526	0.0600	0.0135
MQ–79 HH	Yes	0.0200	0.0041	3.875	0.1109	0.6000	0.1035	0.1450	0.0558
MQ–79 I	Yes	0.0375	0.0048	4.150	0.1658	1.0200	0.1779	0.1575	0.0309
MQ–79 II	Yes	0.0225	0.0025	3.950	0.1848	1.0375	0.2345	0.1000	0.0220
MQ–79 J	Yes	0.0275	0.0048	4.325	0.3146	1.0275	0.1005	0.0925	0.0354
MQ–79 JJ	Yes	0.0150	0.0029	3.950	0.1190	0.6275	0.0602	0.0950	0.0253

[a]See table 6.2 for description of cottons and treatments.
[b]Values are percent reducing substances (sugar content).
[c]Reported as micromhos.
[d]Reported as percent BWE determined from weight loss of the sample.
[e]Values are percent of original sample.
Source: Berni et al. (*10*).

Table 6.4—Discriminant Analysis Using Conductance Classification Results for Calibration Data

Cotton and treatment[a]	From wash group	Classified into wash group	Posterior probability of membership in wash groups: No	Yes
79–1A	No	No	1.0000	0.0000
79–1D	Yes	Yes	0.0000	1.0000
MQ–79 A	No	No	0.9995	0.0005
MQ–79 B	No	No	1.0000	0.0000
MQ–79 C	Yes	Yes	0.0000	1.0000
MQ–79 CC	Yes	Yes	0.0000	1.0000
MQ–79 D	No	No	1.0000	0.0000
MQ–79 E	Yes	Yes	0.0000	1.0000
MQ–79 EE	Yes	Yes	0.0000	1.0000
MQ–79 G	Yes	Yes	0.0000	1.0000
MQ–79 GG	Yes	Yes	0.0000	1.0000
MQ–79 H	Yes	Yes	0.0000	1.0000
MQ–79 HH	Yes	Yes	0.0000	1.0000
MQ–79 I	Yes	Yes	0.0000	1.0000
MQ–79 II	Yes	Yes	0.0000	1.0000
MQ–79 J	Yes	Yes	0.0000	1.0000
MQ–79 JJ	Yes	Yes	0.0000	1.0000

	Number of observations and percents classified into wash group		
From wash group	No	Yes	Total
No	4	0	4
	100.00	0.00	100.00
Yes	0	13	13
	0.00	100.00	100.00
Total	4	13	17
Percent	23.53	76.47	100.00

[a]See table 6.2 for description of cottons and treatments.
Source: Berni et al. (*10*).

Table 6.5—Discriminant Analysis Using Percent Ash Classification Results for Calibration Data

Cotton and treatment[a]	Posterior probability of membership in wash groups			
	From wash group	Classified into wash group	No	Yes
79–1A	No	No	0.9742	0.0258
79–1D	Yes	Yes	0.0001	0.9999
MQ–79 A	No	No	0.9606	0.0394
MQ–79 B	No	No	1.0000	0.0000
MQ–79 C	Yes	Yes	0.0011	0.9989
MQ–79 CC	Yes	Yes	0.0001	0.9999
MQ–79 D	No	No	1.0000	0.0000
MQ–79 E	Yes	Yes	0.0002	0.9998
MQ–79 EE	Yes	Yes	0.0002	0.9998
MQ–79 G	Yes	Yes	0.0000	1.0000
MQ–79 GG	Yes	Yes	0.0000	1.0000
MQ–79 H	Yes	Yes	0.0000	1.0000
MQ–79 HH	Yes	Yes	0.0000	1.0000
MQ–79 I	Yes	Yes	0.0000	1.0000
MQ–79 II	Yes	Yes	0.0000	1.0000
MQ–79 J	Yes	Yes	0.0000	1.0000
MQ–79 JJ	Yes	Yes	0.0000	1.0000

	Number of observations and percents classified into wash group		
From wash group	No	Yes	Total
No	4	0	4
	100.00	0.00	100.00
Yes	0	13	13
	0.00	100.00	100.00
Total	4	13	17
Percent	23.53	76.47	100.00
Prior's	0.5000	0.5000	1.00

[a]See table 6.2 for description of cottons and treatments.
Source: Berni et al. (*10*).

Table 6.6—Discriminant Analysis Using Water Soluble Reducing Substances Classification Results for Calibration Data

Cotton and treatment[a]	From wash group	Classified into wash group	Posterior probability of membership in wash groups: No	Yes
79–1A	No	No	1.0000	0.0000
79–1D	Yes	Yes	0.0000	1.0000
MQ–79 A	No	Yes[b]	0.2197	0.7803
MQ–79 B	No	No	1.0000	0.0000
MQ–79 C	Yes	Yes	0.0000	1.0000
MQ–79 CC	Yes	Yes	0.0000	1.0000
MQ–79 D	No	No	1.0000	0.0000
MQ–79 E	Yes	Yes	0.0000	1.0000
MQ–79 EE	Yes	Yes	0.0000	1.0000
MQ–79 G	Yes	Yes	0.0000	1.0000
MQ–79 GG	Yes	Yes	0.0000	1.0000
MQ–79 H	Yes	Yes	0.0000	1.0000
MQ–79 I	Yes	Yes	0.0000	1.0000
MQ–79 II	Yes	Yes	0.0000	1.0000
MQ–79 J	Yes	Yes	0.0000	1.0000
MQ–79 JJ	Yes	Yes	0.0000	1.0000

	Number of observations and percents classified into wash group		
From wash group	No	Yes	Total
No	3	1	4
	75.00	25.00	100.00
Yes	0	13	13
	0.00	100.00	100.00
Total	3	14	17
Percent	17.65	82.35	100.00

[a]See table 6.2 for description of cottons and treatments.
[b]Misclassified observation.
Source: Berni et al. (*10*).

Table 6.7—Discriminant Analysis Using Boiling-Water Extractables Classification Results for Calibration Data

Cotton and treatment[a]	Posterior probability of membership in wash groups			
	From wash group	Classified into wash group	No	Yes
79–1A	No	No	0.9989	0.0011
79–1D	Yes	Yes	0.0313	0.9687
MQ–79 A	No	Yes[b]	0.1192	0.8808
MQ–79 B	No	No	1.0000	0.0000
MQ–79 C	Yes	Yes	0.0023	0.9977
MQ–79 CC	Yes	Yes	0.0001	0.9999
MQ–79 D	No	No	1.0000	0.0000
MQ–79 E	Yes	Yes	0.0001	0.9999
MQ–79 EE	Yes	Yes	0.0001	0.9999
MQ–79 G	Yes	Yes	0.0006	0.9994
MQ–79 GG	Yes	Yes	0.0000	1.0000
MQ–79 H	Yes	Yes	0.0002	0.9998
MQ–79 HH	Yes	Yes	0.0000	1.0000
MQ–79 I	Yes	Yes	0.0006	0.9994
MQ–79 II	Yes	Yes	0.0007	0.9993
MQ–79 J	Yes	Yes	0.0006	0.9994
MQ–79 JJ	Yes	Yes	0.0000	1.0000

	Number of observations and percents classified into wash group		
From wash group	No	Yes	Total
No	3	1	4
	75.00	25.00	100.00
Yes	0	13	13
	0.00	100.00	100.00
Total	3	14	17
Percent	17.65	82.35	100.00

[a]See table 6.2 for description of cottons and treatments.
[b]Misclassified observation
Source: Berni et al. (*10*).

Table 6.8—Discriminant Classification Criteria

Analysis	Critical classification criteria[a]
Conductance	< 12.56 Cotton classified as washed
Ash	< 0.545 Cotton classified as washed
Reducing substances	< 0.147 Cotton classified as washed
Boiling water extractables	< 1.84 Cotton classified as washed

[a]Criteria were derived from discriminant analyses that assumed equal group variances.
Source: Berni et al. (*10*).

Table 6.9—Data Available for Regression Analyses

Cotton and treatment[a]	Wash group	Reducing substances	Conductance	BWE	Ash	ΔFEV_1
MQ–79 A	No	0.1375	15.475	1.6225	0.6550	−4.4
MQ–79 B	No	0.3575	27.550	3.6350	1.0025	−1.2
MQ–79 C	Yes	0.0350	4.450	1.1750	0.3100	−0.9
MQ–79 D	No	0.2550	23.450	3.2400	1.3400	−4.0
MQ–79 E	Yes	0.0450	3.900	0.8550	0.2425	−1.0
MQ–79 G	Yes	0.0350	4.500	1.0325	0.1975	−2.2
MQ–79 H	Yes	0.0300	3.975	0.8775	0.0600	−1.1
MQ–79 I	Yes	0.0375	4.150	1.0200	0.1575	−1.4
MQ–79 J	Yes	0.0275	4.325	1.0275	0.0925	−0.8

[a]See table 6.2 for description of cottons and treatments.
Source: Berni et al. (*10*).

other cottons, because in this study the data used to calibrate the model were the same data used to test the model. This approach has the effect of biasing the "accuracy" of the model in a positive direction. On the basis of the obtained results, it appears that the four tests discriminate among washed and unwashed cottons. Given a larger data base, a discriminant model involving two or more variables would produce a more accurate prediction model than a model based on a single variable. A two-variable model was not tested on these data because the one-variable model had demonstrated 100 percent correct classification.

Data are shown in table 6.9 for ΔFEV_1 responses from subjects exposed to washed and unwashed cottons of nine samples. Mean assay values for the four tests were taken from table 6.3. The squared multiple correlation coefficients (R^2) were calculated for one- and two-variable models. The square of the multiple correlation coefficient ($\times 100$) is the percentage of variability in the ΔFEV_1 values accounted for by variables in the regression model. These R^2 values are used to find models with the most variation in ΔFEV_1 values.

Data in table 6.10 indicate that a two-variable model of WSRS and CON accounts for 85 percent of the variation, and BWE and ASH, for 71 percent. The method of sample reuse (*14*), which gives less biased estimates of the goodness-of-fit of regression models, substantiates that these two models can be used to predict ΔFEV_1 as shown in tables 6.11 and 6.12.

Table 6.10—Regression Models for Dependent Variable ΔFEV_1

R^2	Variables in model
0.1421	WSRS
0.1473	BWE
0.2581	CON
0.3882	ASH
0.1473	WSRS BWE
0.4454	CON ASH
0.5288	CON BWE
0.5897	WSRS ASH
0.7105	BWE ASH
0.8514	WSRS CON

Source: Berni et al. (*10*).

Table 6.11—Multiple Linear Regression With CON and WSRS as Independent Variables

Source	DF	Sum of squares	Mean square	*F* value	PR > *F*
Model	2	12.89821770	6.44910885	17.19	0.0033
Error	6	2.25067119	0.37511187		
Corrected total	8	15.14888889			

Parameter	Estimate	T for H_o: Parameter = 0	PR > T	Standard error of estimate
Intercept	0.03528832	0.09	0.9311	0.39148226
CON	−0.75671230	−5.35	0.0017	0.14138720
WSRS	54.30174600	4.89	0.0027	11.09356794

Source: Berni et al. (*10*).

Table 6.12—Multiple Linear Regression With BWE and Ash as Independent Variables

Source	DF	Sum of squares	Mean square	*F* value	PR > *F*
Model	2	10.76383238	5.38191619	7.36	0.0243
Error	6	4.38505651	0.73084275		
Corrected total	8	15.14888889			

Parameter	Estimate	T for H_o: parameter = 0	PR > T	Standard error of estimate
Intercept	−2.31426530	−3.58	0.0117	0.64655501
BWE	2.08709306	2.58	0.0415	0.80745885
Ash	−6.50724712	−3.42	0.0142	1.90444761

Source: Berni et al. (*10*).

Domelsmith et al. (*15, 16*) reported recently that potassium, the most predominant metallic element in (unwashed) cotton (*1*), is the most reliable marker for washed vs. unwashed cotton. It accounts for approximately 0.5 percent of the weight of raw cotton and is found in the leaf, bract, stem, and seedcoat for which it accounts for 1-2 percent of the elemental content. In addition, the pericarp has an even higher content of potassium, approximately 3 percent. Three different analysis schemes were used to determine the potassium contents in the washed and unwashed cottons: a potassium sensitive electrode, a spot test, and atomic absorption spectra.

The potassium sensitive electrode used was an Orion model 93–19 with a reference electrode (Orion model 90–02–00) connected to a voltmeter (Cole-Parmer Digiphase or Orion model 801A) with magnetic stirring of the sample. Standards in the 10^{-5} to 10^{-3} M range were prepared by diluting 0.1 M KCl (Orion) with 0.02 percent Triton X-100 in Milli-Q water, followed by addition of 1 ml of 1.0 M NaCl to each 100 ml of standard. The blank contained the same materials as the standard except for the KCl. Cotton samples (0.500 g) were placed in labeled 150 ml beakers followed by addition of 100 ml of 0.02 percent Triton X-100 with stirring to ensure thorough wetting of the fibers. The mixture, covered with a watchglass, was allowed to stand for 30-60 minutes at room temperature. After this waiting period, 1 ml of 1.0 M NaCl was added with stirring, excess solution was pressed from the fibers, and the sample was removed before the electrodes were placed in the test solutions. Results are shown in table 6.13.

To facilitate rapid screening of cottons at the mill level, a spot test for potassium was adapted from the procedure of F. Feigl (*17*), in which a 0.10 g sample of cotton is ignited on a watchglass. After washing the resulting ash with 1 ml of 10 percent acetic acid in deionized water, the suspension that was formed was transferred to a black spot plate and 0.01–0.02 g of sodium cobaltinitrite added. A blank in a nearby well of the same spot plate was included for visual comparison. The spot test was performed on 26 unwashed and 25 washed samples. All washed samples and 10 percent acetic acid blanks gave consistently negative tests; all unwashed samples, including 1 mechanically cleaned and 10 that were weathered by rain, gave consistently positive tests.

The atomic absorption studies were performed on the same solutions and standards used previously for the potassium electrode studies. A Perkin-Elmer Model 380 Atomic Absorption Spectrometer equipped with a hollow cathode potassium lamp was used to analyze the solutions at 766 nm. To suppress potassium ionization in the flame, CsCl was added to give 1000 μg of Cs^+ per 1 ml of test solution; 0.02 percent Triton X-100 was used to make the necessary dilutions.

As expected, the data in table 6.13 indicate that most of the potassium in the cotton was present as water soluble salts so that the free potassium ion concentration of the aqueous extract, measured by the electrode method, was approximately equal to the total potassium, as measured by atomic absorption of the aqueous extracts and the X-ray fluoresence of the cotton fibers. Measurements of potassium ion concentration with respect to duration of extraction indicated that the salts were extracted very rapidly. As a result of these studies, the potassium content of cotton has been shown to be an excellent marker for washed cotton and an indicator of the effectiveness of the water wash throughout a cotton batch.

Statistical relationships between ΔFEV_1 values from the human panel acute exposure studies and the levels of potassium in combination with water soluble reducing substances in the cotton samples were reported recently (*16*). Potassium sensitive electrode studies were performed on four replicates of 17 different cottons from the MQ–79 series of washed and unwashed plus a medical quality cotton and a randomly chosen unwashed cotton. Results of the double-blind study are shown in table 6.14.

Statistical analyses were performed on percent potassium, percent conductance, percent boiling water extractables, percent water soluble reducing substances, percent ash, wash status, and ΔFEV_1. For this set of cottons, the potassium content was highly correlated with conductance ($r = 0.998$, $n = 17$) and also correlated with boiling water extractables ($r = 0.959$, $n = 17$), water soluble reducing substances ($r = 0.967$, $n = 17$), and ash ($r = 0.944$, $n = 17$). Pooled within class correlations indicate that within the washed cotton set and within the unwashed cotton set the following correlations hold for the MQ–79 data set: percent potassium is still highly correlated with conductance ($r = 0.997$) but is less correlated with boiling water extractables ($r = 0.871$), water soluble reducing substances ($r = 0.737$), and ash ($r = 0.647$). The percent potassium, like the percent ash and conductance, correctly classified all of the cotton samples into washed and unwashed sets. Of the five variables in this data set, percent potassium was the best indicator as to whether a cotton sample was washed or not. There was a larger difference in percent potassium content between washed and unwashed cottons than there was for conductance, percent ash, percent boiling water extractables, and percent water soluble reducing substances. This observation is consistent with the relative changes in total chemical composition between unwashed and washed cotton samples. The washing process removes almost all of the potassium but removes porportionately less of the calcium, magnesium, and aluminum compounds which, like potassium, are major contributors to the ash and presumably also major contributors to conductance.

Correlations between ΔFEV_1 data available from the human panel acute exposure studies at Clemson, South Carolina, and these five chemical variables were also explored. The correlation between percent potassium and ΔFEV_1 is low ($r = 0.535$, $n = 9$). The ash content serves as

Table 6.13—Comparison of Potassium Content of Unwashed and Washed Cotton

Cotton and treatment	Potassium content (%), as measured by: Potassium-sensitive electrode[a]	Atomic absorption[b]	X-Ray fluorescence[c]
79–1A, unwashed DPL–55 Stoneville, MS	0.400 ± 0.020[d]	0.420 ± 0.020[d]	0.450
MQ–79 A, unwashed DPL–61 Washington Co., MS	0.360 ± 0.010	0.390 ± 0.020	0.430
MQ–79 B, unwashed Acala SJ–5 Madera Co., CA	0.680 ± 0.040	0.670 ± 0.020	0.650
MQ–79 D, unwashed GSA–71 Lubbock Co., TX	0.530 ± 0.020	0.560 ± 0.010	0.580
MQ–79 C, washed with finish (from MQ–79 B)	0.014 ± 0.002	0.016 ± 0.002	0.010
MQ–79 CC, washed without finish (from MQ–79 B)	0.017 ± 0.001	0.020 ± 0.001	0.021
MQ–79 E, washed with finish (from MQ–79 D)	0.009 ± 0.001	0.009 ± 0.001	0.004
MQ–79 EE, washed without finish (from MQ–79 D)	0.013 ± 0.003	0.015 ± 0.004	0.019
MQ–79 G, washed with finish (from MQ–79 A)	0.015 ± 0.002	0.018 ± 0.003	0.018
MQ–79 GG, washed without finish (from MQ–79 A)	0.013 ± 0.002	0.014 ± 0.002	0.013

[a]n = 5 for 79–1A and MQ–79 A, B, and D; n = 4 for MQ–79 C, CC, E, EE, G, and GG. Values reflect free potassium ion concentration.
[b]n = 4. Values reflect total potassium concentration.
[c]n = 1. Values reflect total potassium concentration.
[d]Mean ± standard deviation.
Source: Domelsmith and Berni (*15*).

Table 6.14—Potassium Selective Electrode Analyses of MQ–79 Series Cottons in Double-Blind Study

Cotton and treatment[a]	Potassium (%)[b]
79–1A	0.41 ± 0.02
79–1D	0.056 ± 0.005
MQ–79 A	0.36 ± 0.02
MQ–79 B	0.66 ± 0.01
MQ–79 C	0.019 ± 0.004
MQ–79 CC	0.015 ± 0.001
MQ–79 D	0.54 ± 0.01
MQ–79 E	0.0098 ± 0.0015
MQ–79 EE	0.013 ± 0.001
MQ–79 G	0.021 ± 0.005
MQ–79 GG	0.012 ± 0.002
MQ–79 H	0.0080 ± 0.0002
MQ–79 HH	0.0056 ± 0.0005
MQ–79 I	0.010 ± 0.002
MQ–79 II	0.0076 ± 0.0023
MQ–79 J	0.015 ± 0.005
MQ–79 JJ	0.0079 ± 0.0026
Medical-grade cotton	0.0070 ± 0.0034

[a]See table 6.2 for description of cottons and treatments.
[b]Mean ± standard deviation.
Source: Domelsmith et al. (*16*).

a slightly better single predictor for ΔFEV_1 for this data set of washed and unwashed cottons. However, its correlation with ΔFEV_1 is also low ($r = 0.623$, $n = 9$). Regression models for ΔFEV_1 employing two independent variables give better correlations for this limited data set. The best predictors of ΔFEV_1 are: 1) percent water soluble reducing substances and percent potassium ($r = 0.926$, $n = 9$), and 2) percent water soluble reducing substances and conductance ($r = 0.923$, $n = 9$). Thus, for these correlations, conductance and potassium content are essentially equivalent.

Microscopical Studies

Rollins et al. (*18*) showed by microscopical techniques that natural surface materials (waxes, sugars, etc.) on the cotton fiber surface could be removed or redistributed during washing, depending on washing conditions. Recently, Goynes et al. (*19*) used transmission and scanning electron microscopy to study changes in surface coating and differences in number of particles present on fibers washed to remove dusts. The MQ–101 series from the Clemson studies were used in this work. Fiber surfaces were studied both directly in the scanning electron microscope (SEM) and in the transmission electron microscope (TEM) by a surface replication procedure. The TEM procedure is more tedious and time consuming than the SEM procedure; however, it provides better resolution of exposed fibrils on the fiber surface.

Washing treatments for the MQ–101 series did not remove surface coating materials. However, microscopical evidence indicated that in some instances the coatings were disturbed enough to change the character of the fiber surface. Figure 6.1a shows a TEM replica of an unwashed, natural cotton fiber and figure 6.1b shows the SEM image of an unwashed fiber. Natural ridges and compression marks as well as some particles attached to the surface can be seen in these micrographs. Some coating material was removed from the surface when fibers were washed at 200 °F (figure 6.2). The surface has a roughened, grainy appearance probably due to the network of the primary wall showing through the thinned coating. However, not all of the fibers examined had this graininess, indicating nonuniformity in removal of the coating.

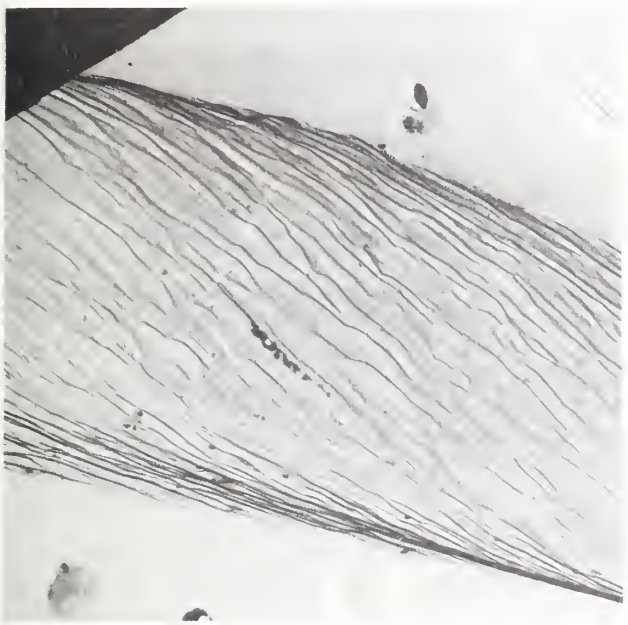

Figure 6.1a—TEM surface replica of natural surface of a cotton fiber.

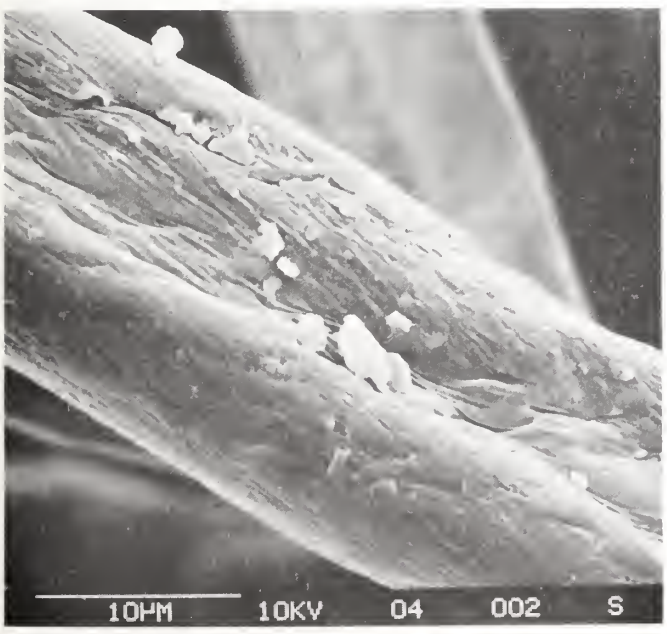

Figure 6.1b—SEM image of natural surface of a cotton fiber.

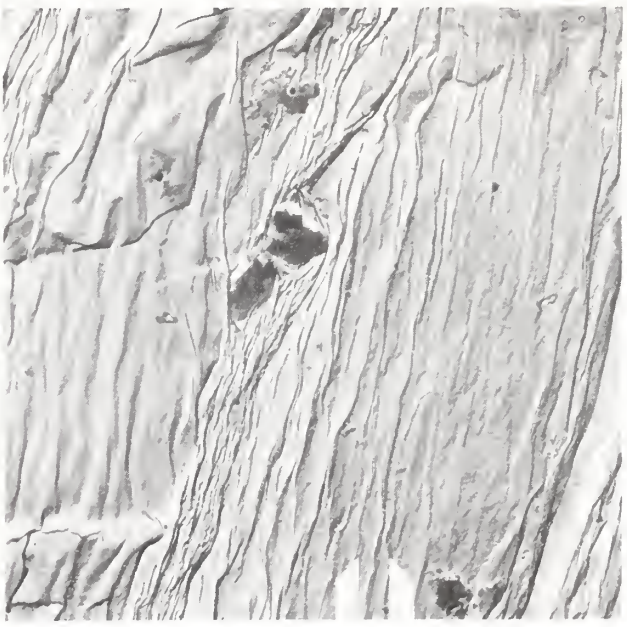

Figure 6.2—TEM surface replica of a fiber washed at 200 °F. (Arrow indicates grainy area.)

In this same sample, surfaces of approximately half of the fibers viewed were splotched with clusters of "soft" particulates fused on to the surface. These particles were different from the trash particles on the surfaces of unwashed cotton. These "soft" particles are shown in figure 6.3. Goynes et al. (*19*) suggested three probable sources for the particles: redeposition of coating materials removed from other areas of the fiber; clumping of finishing material added in the last bath of the washing process; or deposits from fungi growing on the fiber. The authors concluded that the surface changes noted in areas both where fibrils were exposed and where excess materials were deposited could account for the changes in natural processing properties. In addition, all fibers examined by TEM or SEM contained fewer surface particles, and the removal of these particles probably accounts for the reduction of lung dysfunction in human subjects exposed to processing dusts from these washed cottons.

The relationship of contact angle and wettability of materials is well known (*20, 21*). Contact angle decreases with increased wettability. It seemed reasonable to expect that washing of cotton with water would remove sufficient material to change the wettability as measured by contact angle.

The contact angle is defined as the angle between the solid surface and the tangent to the liquid surface as it approaches the solid, the angle being measured in the liquid as shown in figure 6.4. Equilibrium contact angles were

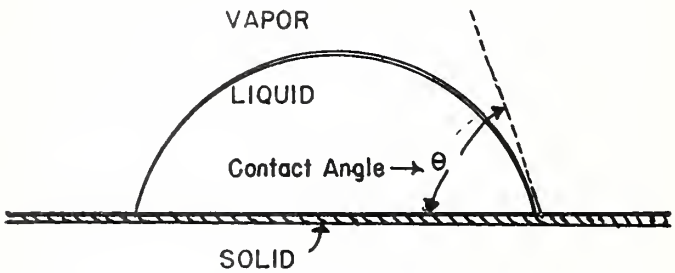

Figure 6.4—Schematic of contact angle between drop of liquid and solid surface.

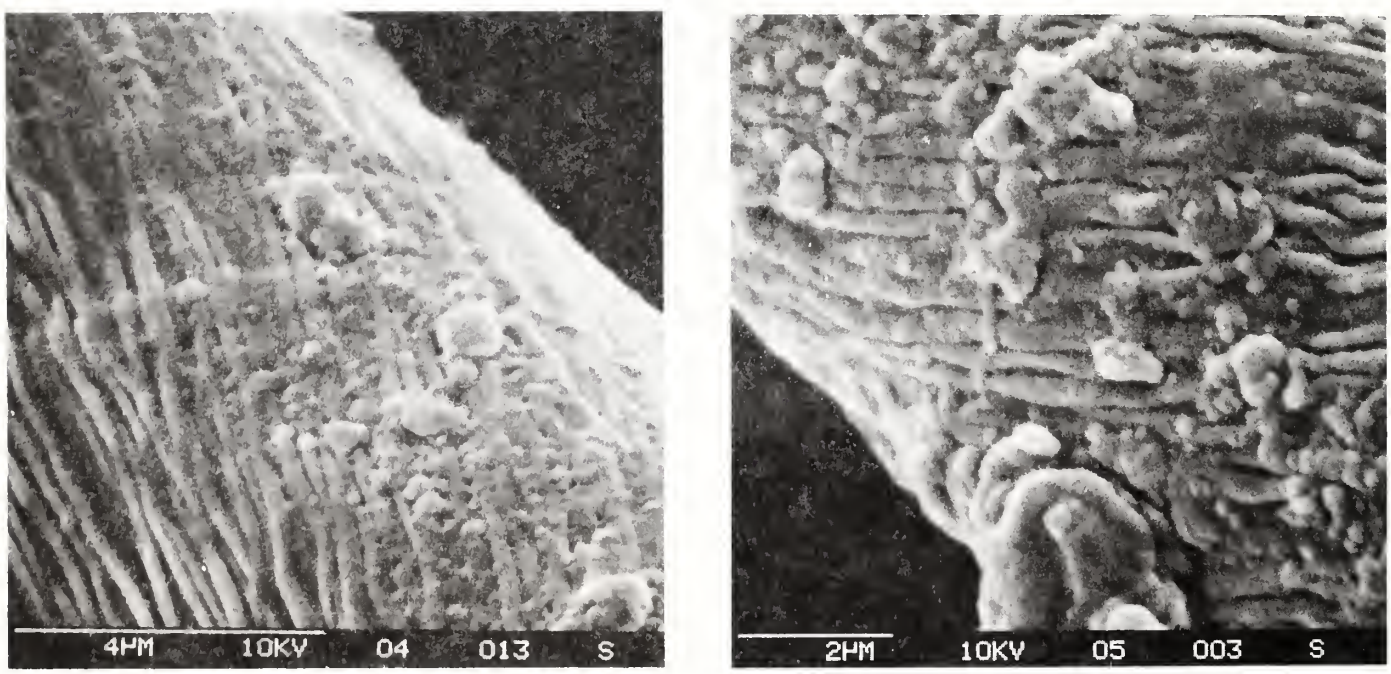

Figure 6.3—SEM of two fibers washed at 200 °F showing presence of soft particulate coating.

measured on a layer of combed fibers by use of a Ramé-Hart contact angle goniometer. Fibers were combed with a pair of stainless steel combing tools until a layer of parallel fibers was formed. The layer of fibers was placed on the goniometer stage so that the fibers were parallel to the line of sight of the goniometer telescope. This prevents capillary action in the grooves between fibers from distorting the shape of the drop of liquid in a plane perpendicular to the telescope line of sight. A sessile drop of water approximately 2.5 mm in diameter was deposited onto the fibers by use of a microsyringe attachment furnished with the goniometer. Six replicates for each sample were measured, and the arithmetic mean was taken as the contact angle for a particular sample. The standard deviation of the procedure was 2.72 degrees.

Table 6.15 shows the effects of water washing on contact angles of fibers with water. Although one of the unwashed samples and two of the washed samples had standard deviations greater than that of the method, these values are included for comparative purposes. Contact angles for the four samples decreased after washing. This would be expected—the cleaner the surface, the more readily the liquid spreads. There does not seem to be a relationship between percentage of change in the contact angle caused by washing and the locations at which the cotton was grown. However, cotton grown in the eastern part of the Nation had a higher contact angle. This indicates that there is a greater concentration of hydrophobic material in, or on, the primary wall of cotton grown in eastern areas of the United States (*22*). Dust storms, rainfall, harvesting procedure, and humidity vary with the location of growth. Variety could also affect chemical composition and hence the contact angle. Two varieties grown in the same State showed almost identical contact angles before washing, but the percentages of change due to washing were considerably different. The high values obtained may be explained by wax on the fiber surface. The contact angle would be expected to be about 110° between a paraffin wax and water.

Pittman (*23*) used a sink-float technique to study the wetting behavior of wool and mohair. This technique, developed by Mutchler et al. (*24*), is based on the principle that when the critical surface tension (CST) of a fiber snippet about 5 mm long is greater than the surface tension of a liquid, the fiber will be completely wetted and sink. If the CST of the fiber is less than the surface tension of the liquid, the fiber will not wet and will float. The surface tension at which the unwashed cotton floats is between that of tetrabromethane (47.5 dynes/cm) and that of 20 percent dioxane in water (51.57 dynes/cm). Below this, the surface tension of the cotton is equal to, or greater than, that of the liquid so the cotton wets and sinks. It was found that either 20 percent dioxane in water or 1 percent acetone in water could be used as the liquid in a sink-float test to differentiate between washed and unwashed cotton fibers. A difficulty with this test is that a small amount of surfactant on the fibers might cause the fibers to sink, thus passing the test even though they had not been washed.

Table 6.15—Effect of Water Wash[a] on Contact Angle

Area of growth and variety[b]	Equilibrium contact angle between H_2O and cotton (degrees)[c]				
	Unwashed	SD	Washed	SD	Change (%)
Mississippi, DPL–55	127	1.633	110	2.380	−13.39
Mississippi, DPL–61	130	4.472	103	3.764	−20.76
Texas, GSA–71	118	1.732	108	1.732	− 8.47
California, Acala SJ–5	107	2.582	83	4.796	−22.43

[a]Washed on commercial rayon rinse system at 66 °C, 56:1 water: cotton weight ratio.
[b]Samples from MQ–79 series.
[c]Arithmetic mean of 6 duplicates of same sample.

Mack and Donaldson (*25*) showed that when inorganic salts were added to purified cotton the pyrolysis profile changed. Even small concentrations of basic salts caused a reversal from the usual endotherm observed with cotton to an exotherm. It has been known since 1924 (*26*) that inorganic compounds alter the pyrolysis of cotton, generally lowering the onset pyrolysis temperature, decreasing the amount of tar attributed to β-levoglucosans, and increasing the amount of solid residue.

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were investigated as techniques for thermal analyses of washed and unwashed cottons (*27*). The apparatus used was a Du Pont 1090 Thermal Analysis System (Du Pont Analytical Instruments Division, Du Pont Co., Wilmington, Delaware). The control unit combines a digital temperature programmer, visual display, disk memory, data analyzer, and a printer/plotter. Du Pont 910 DSC and 951 TGA modules were plugged into the control unit. This equipment allows for gathering, recalling, analyzing, and plotting data.

For DSC, 5 mg samples were used; for TGA, 10 mg samples were used. Samples were ground in a Wiley mill to pass a 20 mesh screen and a weighed amount pressed into a disk under 2000 psi in a hydraulic press. The press was a Pasadena Hydraulic, Inc. Model P–21 of a type used to make potassium bromide disks for instrumental analyses. Heating rates of 15 °C per minute were used for both DSC and TGA. All analyses were made with dry nitrogen as a sweep gas. Earlier work with differential thermal analysis (DTA) on textile materials (*28–30*) had shown that results are highly reproducible with respect to features and the temperatures at which transformations occur. The curves are unique for a given composition. Certain precautions, such as occasional temperature standardization and maintaining the same heating rate, are necessary to achieve a high degree of reproducibility.

Figure 6.5 contains DSC and TGA thermograms for a purified cotton. The DSC shows an initial large endotherm for moisture loss. This event shows as a slight weight loss in TGA. Both DSC and TGA show substantial changes at about 360 °C. DSC shows a substantial endotherm, and TGA shows a significant loss of weight. This has been attributed to thermal scission of cellulose chain to form levoglucosans.

Figure 6.6 contains DSC thermograms of unwashed cottons grown in Mississippi, Texas, and California (MQ–79). All three samples had an initial endotherm associated with moisture loss. This was followed by a shallow endothermic area from about 150 °C to about 300 °C which may be due to decomposition of waxes on the surface of the cotton. At about 350–360 °C there is an exothermic peak for all samples. This peak increases as the growing location of the cotton moves west in the United States. Potassium content follows this same pattern (*16*).

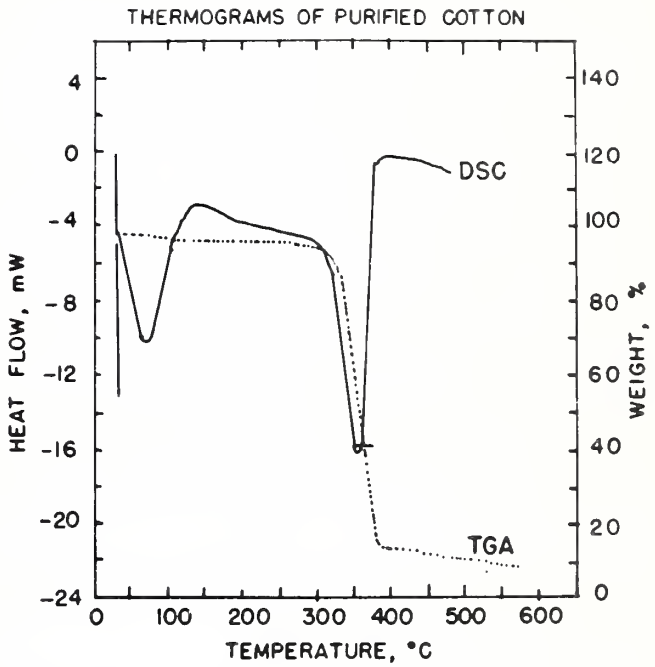

Figure 6.5—DSC and TGA thermograms of purified cotton.

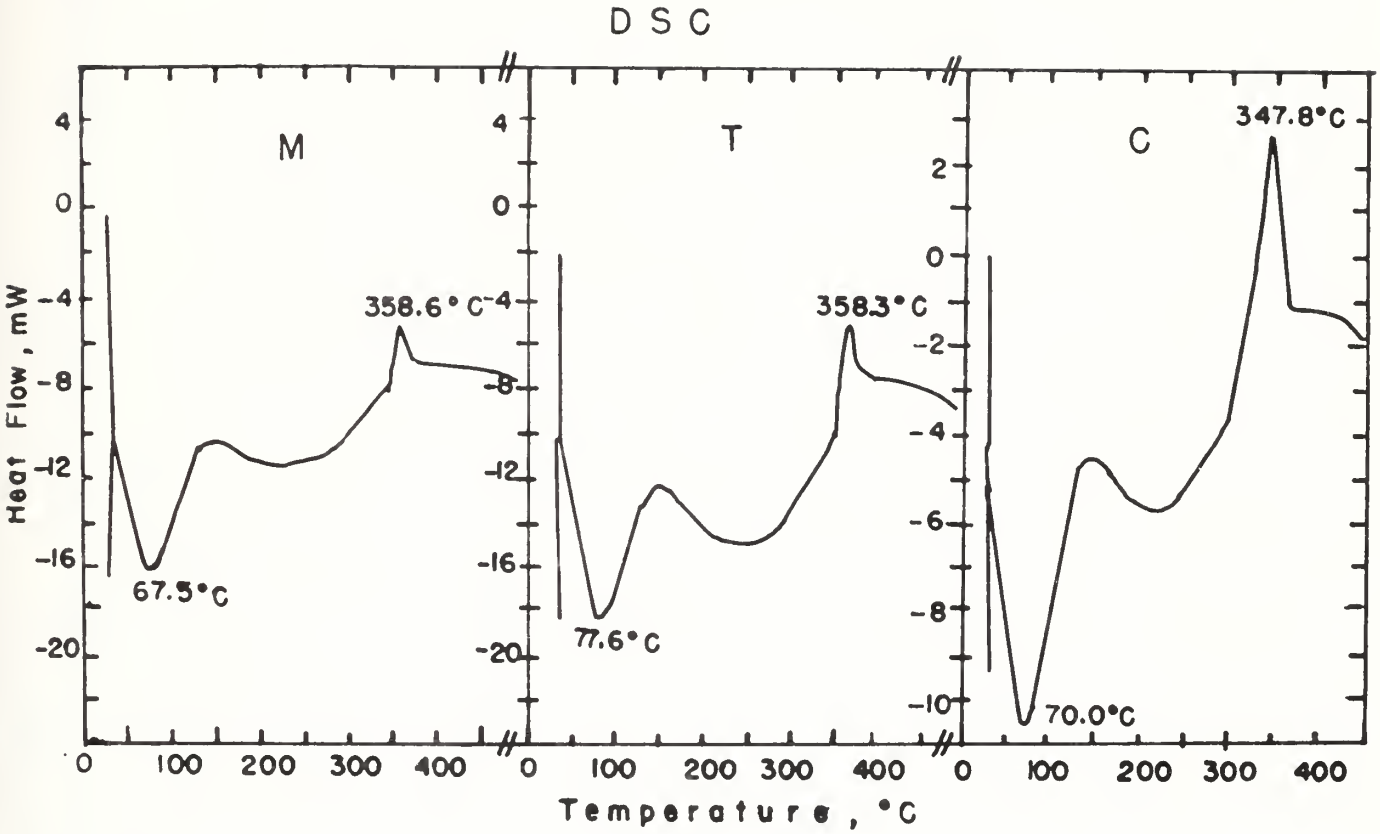

Figure 6.6—DSC thermograms of unwashed cottons (MQ–79 series). M, Mississippi DPL–61; T, Texas GSA–71; C, California Acala SJ–5.

Figure 6.7 contains DSC thermograms of three washed cottons (MQ–79) and a specially cleaned medical-grade (Parke-Davis) control. These washed cottons are the clean analogs of the unwashed cottons in figure 6.6. All of these cleaned cottons have similar thermograms. The California cotton does have two small endotherms at 200.7 °C and 273.0 °C that are not present with the other samples. They are evidently due to material not removed by water washing. These DSC curves indicate that the washed cottons are as "thermally clean" as the medical grade cotton.

Figure 6.8 contains TGA curves for unwashed and washed Mississippi cottons (MQ–79) as well as for medical-grade cotton. The most noticeable difference in the TGA results between washed and unwashed cottons is the percentage of residue remaining at the conclusion of pyrolyses. Unwashed cotton results in about twice as much residue as does either cleaned cotton. In comparing the TGA results with the DSC data, the loss of moisture is endothermic but results in minimal loss of weight. In contrast, the destruction of clean cellulose at 360 °C results in both a large endotherm by DSC and a large weight loss by TGA.

Table 6.16 gives TGA data for three varieties of washed and unwashed cottons (MQ–79) and for medical-grade control. The transition weight loss starts at a lower temperature for the unwashed than for the corresponding washed samples. This difference in onset temperatures is greater than the difference between final temperatures. The major difference is that the percentage of residue for washed samples is about 10 percent whereas that for unwashed samples is about 20 percent.

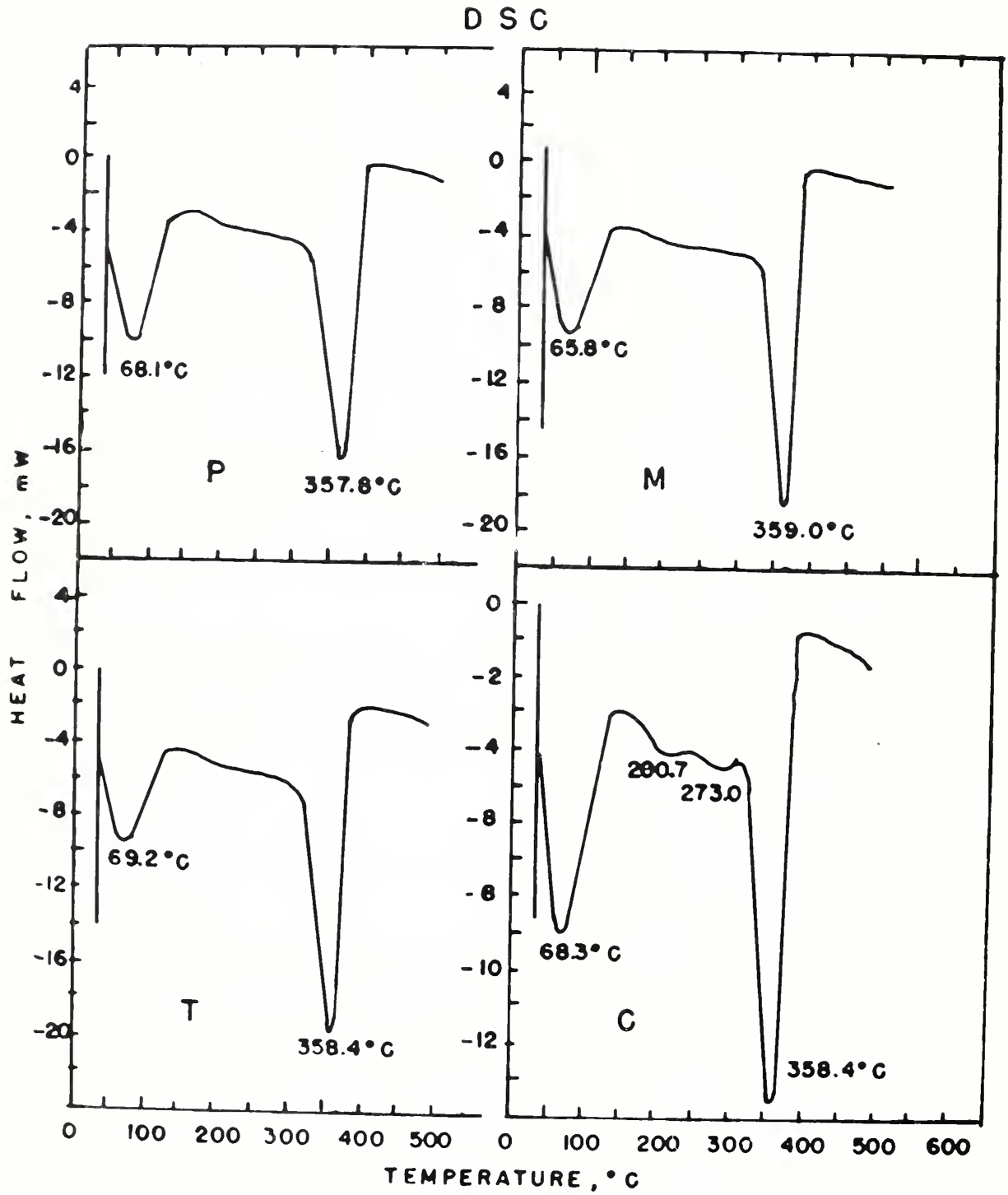

Figure 6.7—DSC thermograms of washed cottons (MQ–79 series). M, Mississippi DPL–61; T, Texas GSA–71; C, California Acala SJ–5; P, purified control cotton.

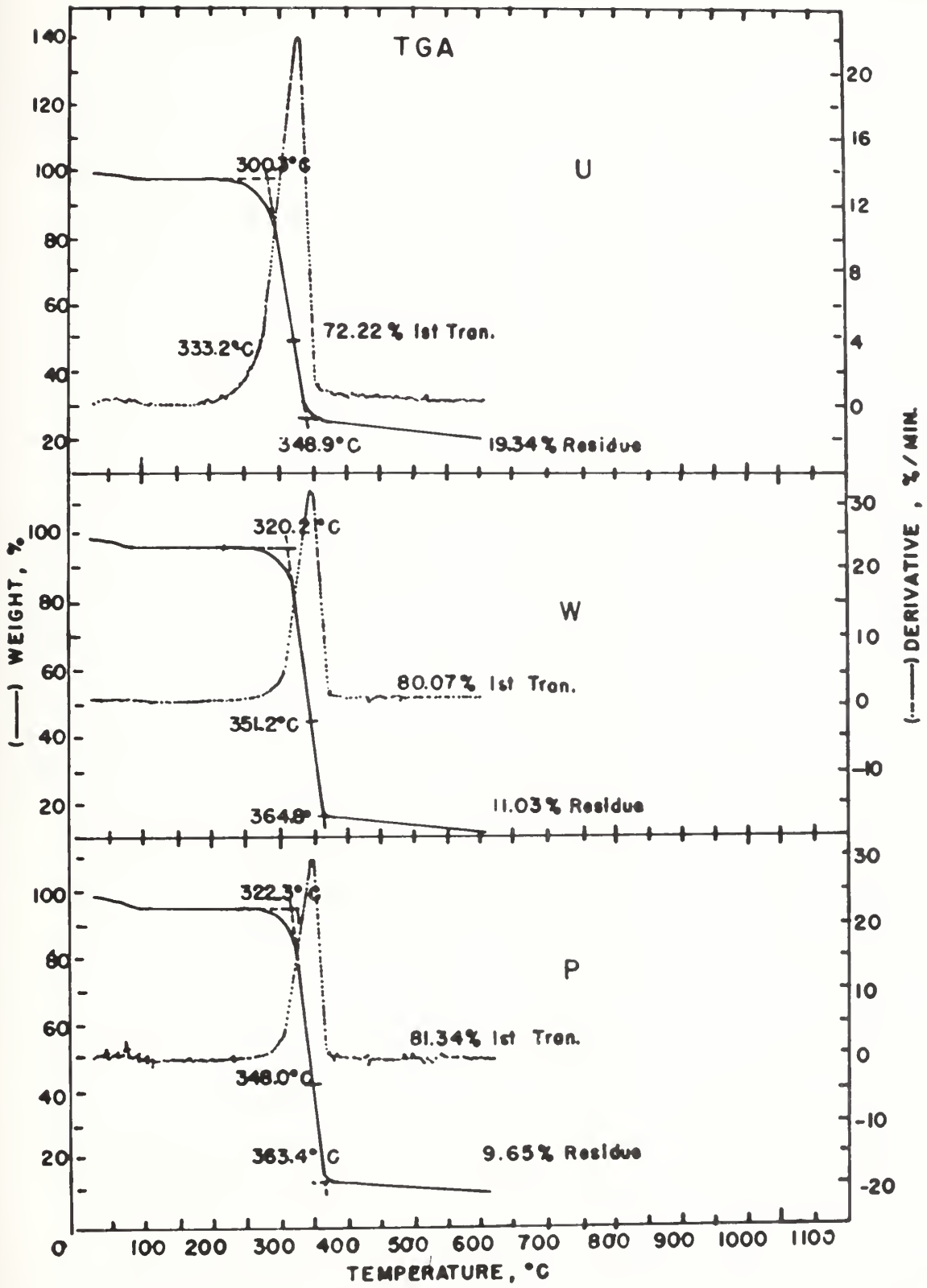

Figure 6.8—TGA thermograms of unwashed and washed Mississippi DPL–61 (MQ–79 series) and purified cottons. U = unwashed; W = washed; P = purified.

Table 6.16—Thermal Gravimetric Analysis[a] Data of 3 Varieties of Cotton

Variety[b]	Temperature of first transition (°C)			Weight loss (%)	Residue (%)
	Start	End	Change		
			Unwashed		
DPL–61	300.3	348.9	48.6	72.22	19.34
GSA–71	290.5	339.9	49.4	72.31	18.59
Acala SJ–5	292.2	341.6	49.4	69.46	19.97
			Washed[c]		
DPL–61	328.2	364.8	36.6	80.07	11.03
GSA–71	329.0	367.5	38.5	79.71	9.53
Acala SJ–5	324.1	364.3	40.2	79.69	11.28
Control[d]	322.3	363.4	41.1	81.34	9.65

[a]Approximately 10 mg sample, 15 degrees per minute.
[b]MQ–79 series.
[c]Commercial rayon rinse system, 66 °C, 56:1 H_2O: cotton weight ratio.
[d]Medical-grade cotton.

Summary and Conclusions

Before "washed cotton" can become a candidate for an exemption from the Dust Standard, the Industry/Government/Union Task Force for Byssinosis Prevention believes that there must be a scientifically acceptable and measurable difference between "washed" and "unwashed" cotton. This study has shown that several analyses can be used to determine whether a cotton sample has been washed or not.

The analyses that have been used successfully to determine the wash status of cotton samples are: conductance, potassium content, contact angle, DSC, TGA, and ash content. In a double-blind study of 13 washed cottons and 4 unwashed cottons from the MQ–79 series, three of the analyses—conductance, potassium content and ash content—were tested and correctly distinguished between washed and unwashed cottons. Since completion of the double-blind studies, potassium contents of 21 unwashed and 14 washed cottons have been determined. The unwashed cottons consistently contained at least six times as much potassium as washed cotton (0.36–0.73 percent vs. 0.00–0.06 percent). Contact angle, DSC, and TGA were investigated after the double-blind study.

Statistical methods were used to probe the relationship between each of five of the test parameters and ΔFEV_1 measured in the human panel acute exposure studies with these same cottons. The five parameters probed were percent ash, percent conductance, percent water soluble reducing substances, percent potassium, and percent boiling water extractables. Regression models employing two parameters led to good correlations with ΔFEV_1. The best predictors of this important lung function measurement for the MQ–79 series of cottons are: 1) water soluble reducing substances and percent potassium ($r = 0.926$, $n = 9$) and 2) water soluble reducing substances and conductance ($r = 0.923$, $n = 9$).

Acknowledgments

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Chapter 7
Other Related Studies—Chemical and Microbiological Composition of Water Washed and Aqueous Acetone Washed Cottons: Human Ventilatory Response to Generated Dusts

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Introduction

Tannins are present in significant quantities in cotton bract, leaf, and dust from cotton plant parts. The quantities of these biologically active substances in cotton dust are sufficiently high to be possible causes of the biological effects associated with byssinosis. Because of this, Bell and Stipanovic suggested that tannins be evaluated as possible etiological agents of byssinosis by either human exposure or animal studies (*1*). So to determine if tannins are involved in the etiology of byssinosis, cotton was washed in a batch system with aqueous acetone for use in human exposure studies and for chemical and microbiological analyses of lint and generated dust (*2*). Aqueous acetone was chosen because tannins are more soluble in that solvent than in water.

Materials and Methods

A mix of high microbiological content cotton (MQ–135) comprising three bales was prepared at the USDA Agricultural Research Services Cotton Quality Research Station (CQRS), Clemson, South Carolina. Three blended bales weighing 363, 355, and 367 pounds (165, 161, and 166 kg) were produced from the mix by processing through six feeder hoppers. Each blended bale contained identical proportions of the three source bales. The bales were shipped to the USDA Agricultural Research Services Southern Regional Research Center (SRRC), New Orleans, Louisiana, for the washing treatments. Each of the bales was opened on the SRRC opening line that consisted of a feeder hopper and Superior Cleaner. One bale was retained as an untreated control. This bale had some extraneous woody trash that came from the blending line at CQRS; therefore, in addition to the hopper feeder and the Superior Cleaner, it was processed through a Rando Cleaner that removed the extraneous trash. The two bales for use in the washing treatments were not processed through the Rando Cleaner. The bales for the washing treatments were divided into 41-pound (19 kg) lots for use in a batch washing system.

The washing treatments were conducted outside the SRRC wet processing pilot plant with elaborate safety precautions to provide adequate ventilation and to prevent explosions. The two washing treatments were: 1) Washaid 1173 (wetting agent) + 70 percent aqueous acetone, water rinse, SSC Finish 641, oven dry (aqueous acetone wash); and 2) Washaid 1173 (wetting agent) + water, water rinse, SSC Finish 641, oven dry (water wash).

The batch system used for these washing treatments was a screened basket container for the cotton that could be lowered into a large metal, cylindrical beck (28 inches (71 cm) diameter, 30 inches (76 cm) in height) capable of holding more than 80 gallons (303 l) of liquid. The washing solution for the aqueous acetone treatment contained 1.33 pounds (0.6 kg) of Washaid 1173, 56 gallons (212 l) of acetone, and 24 gallons (91 l) of water to give a 70 percent acetone solution. The cotton stock and solution were added slowly and simultaneously to the container to aid wetting of the stock. After all of the fiber and solution were added, the mixture was allowed to stand for 1 hour. The solution was then removed from the container by pumping into a 55-gallon (208 l) drum for proper disposal. The fiber batt was rinsed with cold tap water for 15 minutes using two garden hoses to aid transport of solubilized materials and reduce acetone content. After excess liquid was drained from the tank, a new solution containing 4 pounds (1.8 kg) of SSC Finish 641 in 80 gallons (303 l) of water was added to the batt. The cotton was allowed to stand for 1 hour; then the solution was drained by lifting the batt partially out of the container using a hoist. The cotton was centrifuged to a moisture content of about 40 percent and a theoretical SSC Finish 641 level of about 0.4 percent. Approximately one-half of the batch was centrifuged at a time at 1700 g's for 6 minutes. After centrifuging, the cotton was opened by hand

Results and Discussion

and dried on a wire rack in a Proctor-Schwartz oven for 16 hours at 80 °C. The procedure for the water wash treatment was exactly the same as for the aqueous acetone treatment except that 56 gallons (212 l) of water was substituted for the 56 gallons (212 l) of acetone.

Chemical and physical tests were conducted on stock taken at various stages of the washing treatments. Elemental analyses were conducted on the cottons at SRRC. Chemical analyses for water soluble reducing substances (sugars) and amount of finish were conducted at CQRS. Fiber property measurements and spinning quality evaluations were conducted by the usual methods employed at CQRS. Endotoxin contents of the fiber samples and viable microorganisms both in lint samples and in the air of the remote rooms were determined by one or more laboratories using methods reported previously (*3–5*). Endotoxin in airborne dust in the remote rooms was determined by analysis of vertical elutriator filters as reported previously (*6*). Analyses for tannins were conducted at the USDA Agricultural Research Services National Cotton Pathology Research Laboratory, College Station, Texas, by use of reported methods (*1*).

The cottons were processed in model card rooms at CQRS to generate dust for the human exposures in the adjacent remote rooms using human subjects and procedures reported previously (*7*). The cottons were spun into 30s (19.7 mg/m) yarn at 13000 rpm spindle speed. The quantities of cotton were limited because of the complexity, expense, and exploratory nature of the washing procedures, and only one replication was available for the human exposure studies and subsequent processing evaluations through spinning. Except for pulmonary function responses, statistical analyses were not conducted because of the lack of replications; thus, the results should be interpreted with these factors taken into consideration.

Results of the elemental analyses on raw stock samples are shown in table 7.1. Both of the washing treatments reduced the quantities of the elements to essentially the same levels. The most dramatic reduction occurred with potassium, which was reduced from 0.51 percent to 0.03 percent. This is in keeping with results reported by Domelsmith et al. (*8*). This property has been exploited as a potential indicator for verifying washed cotton. Results of elemental analyses on drawing sliver of the same cottons are shown in table 7.2. Compared with raw stock, drawing sliver is well blended and relatively trash free. Generally, the levels of elements were somewhat lower for drawing sliver than for raw stock for both the unwashed and the washed cottons. There were no practical differences in residual levels of elements between washing treatments.

Sugar contents of drawing sliver were 0.33 percent for the unwashed control and 0.16 percent for both the aqueous acetone washed and the water washed cottons. The residual sugar contents of the treated cottons are higher than that found for washed cottons in previous washing trials (*9, 10*). This indicates that the treatments were not as effective in removing water soluble compounds as those used to wash cotton on either the rayon rinse system or the Cotton Incorporated continuous batt system. As noted in earlier commercial scale batch washing trials, channeling of solution can occur, the cotton batt acts as a filter medium, and transport of both insoluble and solubilized impurities is poor (*11*). The finish contents of the two treated cottons were in the 0.3–0.5 percent range as targeted. Because of the variability of the stock sampled before addition of finish for use as a background control, more precise determination could not be made easily.

The fiber properties normally measured—length, strength, micronaire—were not affected by either of the two washing treatments. However, the processing and yarn

Table 7.1—Effects of Washing Treatments on Removal of Selected Elements From Cotton—Raw Stock Samples

	Element quantity (%)		
Element	Before wash (MQ–135 A)[a]	Aqueous acetone wash (MQ–135 B)[a]	Water wash (MQ–135 C)[a]
Nitrogen	0.25	0.20	0.19
Phosphorus	0.14	0.12	0.13
Magnesium	0.06	0.00	0.01
Calcium	0.19	0.15	0.14
Potassium	0.51	0.03	0.03
Sulfur	0.07	0.05	0.05
Chlorine	0.04	0.01	0.01
Silicon	150 ppm	64 ppm	58 ppm

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

qualities were adversely affected by both treatments (table 7.3). Card web neps were the same for the control and the aqueous acetone treatment but higher for the water wash treatment. End breakage in spinning, yarn strength, and yarn appearance factors were adversely affected by the two washing treatments. Comparison of the two washing treatments leads to the conclusion that the aqueous acetone treatment yielded stock that was processed better and produced better yarn than did the stock produced by water washing. Because of the lack of replications, the processing and yarn quality evaluations should be used only as indicators of probable trends.

The endotoxin levels and the viable total and gram-negative bacteria levels on lint samples as determined by the three laboratories are shown in table 7.4. Although reported levels differed between laboratories, the treatments were ranked in the same order by the different laboratories. The two washing treatments reduced both total and gram-negative bacteria by up to 3 logs, with the water wash treatment apparently being more effective. The endotoxin levels were also reduced by the two washing treatments; the aqueous acetone treatment brought about a 10-fold reduction and the water wash treatment a 25–100–fold reduction, depending on which laboratory data are considered. The conclusion is that the bacteria and endotoxin levels are reduced by both washing treatments, but residual levels are higher for the aqueous acetone treatment than for the water wash treatment.

The total and gram-negative bacteria and fungi present in the air of the remote rooms during human exposures are shown in table 7.5. Each of these components was reduced by the washing treatments. Of the two treatments, total bacteria in air appeared lower for the water wash. However, there was little difference, if any, in either gram-negative bacteria or fungi between the two washing treatments. Endotoxins in airborne dusts and concentrations in air of the remote rooms are shown in table 7.6. Both washing treatments significantly reduced the endotoxin levels. The differences in endotoxin levels between the washing treatments were small and may not be of practical significance.

Table 7.2.—Effects of Washing Treatments on Removal of Selected Elements From Cotton—Drawing Sliver Samples

	Element quantity (%)		
Element	Before wash (MQ–135 A)[a]	Aqueous acetone wash (MQ–135 B)[a]	Water wash (MQ–135 C)[a]
Nitrogen	0.19	0.14	0.20
Phosphorus	0.06	0.04	0.02
Magnesium	0.09	0.05	0.03
Calcium	0.16	0.13	0.11
Potassium	0.48	0.03	0.03
Sulfur	0.03	0.01	0.01
Chlorine	0.08	0.03	0.02
Silicon	143 ppm	97 ppm	19 ppm

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 7.3—Processing and Yarn Qualities

				Yarn appearance	
Treatment[a]	Card web neps (no./645 cm^2)	Spinning EDMSH[b]	Yarn break factor (units)	Grade	Index
Unwashed control (MQ–135 A)	10	29	1810	C+	103
Aqueous acetone wash (MQ–135 B)	10	47	1705	C	94
Water wash (MQ–135 C)	16	85	1541	C	94

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Ends down per 1000 spindle hours.

Table 7.4—Bacteria and Endotoxin in Lint

Treatment[a]	Total bacteria (CFU/g)	Gram-negative bacteria (CFU/g) Lab A	Gram-negative bacteria (CFU/g) Lab B	Endotoxin (ng/g) Lab A	Endotoxin (ng/g) Lab C
Unwashed control (MQ–135 A)	3.16×10^6	1.66×10^6	2×10^5	100000	15917
Aqueous acetone wash (MQ–135 B)	3.89×10^5	3.55×10^4	1×10^2	10000	1253
Water wash (MQ–135 C)	5.25×10^4	5.75×10^3	50	1000	637

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 7.5—Bacteria and Fungi in Air of Remote Rooms During Human Exposures

Treatment[a]	Total bacteria (CFU/mg)	Total bacteria (CFU/m^3)	Gram-negative bacteria (CFU/mg)	Gram-negative bacteria (CFU/m^3)	Fungi (CFU/mg)	Fungi (CFU/m^3)
Unwashed control (MQ–135 A)	281000	61900	11900	2610	1960	420
Aqueous acetone wash (MQ–135 B)	17700	3890	1460	322	1300	282
Water wash (MQ–135 C)	11400	2350	1420	292	1570	321
Clean room	—	88	—	11	—	139

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Table 7.6—Endotoxins in Airborne Dusts and Concentrations in Air

Treatment[a]	Endotoxins in dust (ng/mg)[b]	Endotoxins in air (ng/m^3)
Unwashed control (MQ–135 A)	1225.90 ± 73.84	257.39 ± 10.35
Aqueous acetone wash (MQ–135 B)	45.68 ± 5.67	8.98 ± 1.06
Water wash (MQ–135 C)	29.32 ± 1.33	5.93 ± 0.26

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).
[b]Mean ± standard error.

The analyses for tannins were conducted on lint samples from the final stages of the preprocessing treatments. The untreated control sample was stock that had been processed through the SRRC opening line. The aqueous acetone wash sample and the water wash sample were collected after the final treatment rinses and were air dried. The average levels of tannins are shown in table 7.7. The variations in levels of tannins were somewhat high between test replications. However, both washing treatments apparently lower the tannin levels in the cotton. There appears to be little, if any, difference between the two washing treatments in effectiveness in removing tannins.

Results of the human exposures are shown in table 7.8. The observed decrease in mean FEV_1 (forced expiratory volume at 1 second) of 8.4 percent for the panel when exposed to the untreated control is very large considering the low dust level (0.22 mg/m^3). The extreme potency of the dust from this cotton on ventilatory response in human subjects is emphasized by the slope of the dose response linear regression, approximately 45 percent FEV_1 decrement per mg/m^3 of elutriated dust. Both washing treatments greatly reduced the response of the panel to the dust generated during carding. However, the panel responses to dusts from the two washed cottons were still greater than the response to the clean room exposure as indicated by the decrease in FEV_1 of about 2 percent for the washing treatments and an increase in FEV_1 of 0.4 percent for the clean room exposure. Thus, the treatments did not completely remove or neutralize the agent(s) responsible for the acute response. The difference in responses between the two washing treatments was not statistically significant.

Table 7.7—Effects of Washing Treatments on Removal of Tannins From Cotton

Treatment[a]	Tannin content (ppm)		
	Extractable	Residual	Total
Unwashed control (MQ–135 A)	87	60	147
Aqueous acetone wash (MQ–135 B)	14	45	59
Water wash (MQ–135 C)	20	46	66

[a]Designations in parentheses refer to Cotton ID and washing treatment (see app. 2).

Table 7.8—Effects of Washing Treatments on Human Responses to Dust

Treatment[a]	Dust level (mg/m^3)	Mean ΔFEV_1 (%)	Slope ΔFEV_1 (%) per mg/m^3
Unwashed control (MQ–135 A)	0.22	−8.4	−45.4
Aqueous acetone wash (MQ–135 B)	0.22	−2.3	−14.4
Water wash (MQ–135 C)	0.21	−1.8	−13.1
Clean room	0.03	+0.4	—

[a]Designations in parentheses refer to cotton ID and washing treatment (see app. 2).

Conclusion

Washing high microbiological content cottons in a batch system by use of either an aqueous acetone-surfactant formulation or a water-surfactant formulation reduced the levels of tannins, viable total and gram-negative bacteria, and endotoxins in the cotton. The washing treatments reduced the potency of the respirable dust on the ventilatory response of human subjects by about 75 percent but not to the level of no exposure. It was not possible to completely isolate the individual effects of tannins, viable bacteria, or endotoxins on changes in human ventilatory response because the effects of the washing treatments on the residual contents of these materials were not independent. Further experimentation is suggested to determine the independent effects of acetone-water washing treatments on tannins, bacteria, and endotoxins.

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Appendix 1
Clemson Human Exposure Studies (MQ Numbers)

Test No.	*Brief Title*
79–1	Green boll cotton study, 1979 crop
MQ–79	Effect of washing commercial, Mississippi, Texas, and California cotton on the rayon rinse system (1979 crop)
MQ–89	Effect of washing commercial Mississippi cotton on the wool scouring system using three wash bowls
MQ–90	Effect of washing Mississippi cotton on the wool scouring system using five wash bowls (commercial Mississippi cotton except MQ–90C which was MQ–80 cotton)
MQ–91	Comparison of the wool scouring and the batch kier systems (MQ–80 cotton)
MQ–95	Effect of water temperature on washing on the batch kier system (MQ–80 cotton)
MQ–101	Comparison of the batch kier and the continuous batt systems (MQ–80 cotton)
MQ–102	Screening test to select panels of human subjects (MQ–80 cotton)
MQ–107	Effect of batt preparation on the continuous batt system (MQ–80 cotton)
MQ–111	Effect of washing conditions on Mississippi cotton (MQ–80 cotton)
MQ–113	Effect of washing conditions on commercial Mississippi cotton with high gram-negative bacteria content
MQ–135	Effect of acetone/water washing on dust from commercial Mississippi cotton
MQ–139	Effect of washing conditions on commercial Mississippi cotton with high endotoxin levels
MQ–CR	Effect of clean room conditions on human panel response

Appendix 2
Identification of Washed Cotton Treatments

79–1:

All treatments done on the rayon rinse system using a 23-bale mix of DPL–55 cotton.

A — Unwashed control

D — Washed (66 °C, 56:1 water-to-fiber ratio)

MQ–79:

Unless otherwise designated, all treatments done on the rayon rinse system using cottons from three areas of growth.

A — Mississippi DPL–61 cotton (Washington County, Mississippi)—Unwashed

B — California SJ–5 cotton (Madera County, California)—unwashed

C — California washed (66 °C, 50:1 Water-to-fiber ratio)

D — Texas GSA–71 cotton (Lubbock County, Texas)—unwashed

E — Texas washed (66 °C, 50:1 water-to-fiber ratio)

F — Mississippi unwashed (same as MQ–79 A)

G — Mississippi washed (66 °C, 50:1 water-to-fiber ratio)

H — Mississippi scoured (done on wool scouring system, 66 °C)

I — Mississippi washed (28 °C, 65:1 water-to-fiber ratio)

J — Mississippi washed (66 °C, 65:1 water-to-fiber ratio)

MQ–80:

MQ–80 describes a 450-bale quantity of DPL–61 cotton grown in Mississippi. The cotton was harvested and blended for use in a yearlong prospective study of washed cotton. Most of the cotton used for the washing trials is from MQ–80 cotton and is designated as such in this monogram.

MQ–89:

Unless otherwise designated, all cottons were washed on the wool scouring system using cottons from an 18-bale mix of Memphis Territory Mississippi cotton purchased commercially.

A — Unwashed control

B — 79–1D (DPL–55)

C — Washed (27 °C—bales 15, 16), late bales

D — Washed (27 °C—bales 6, 7), early bales

E — Washed (49 °C—bales 4, 5), late bales

F — Washed (49 °C—bales 1, 2), early bales

G — 79–1A (DPL–55)

I — MQ–79 C (SJ–5)

L — MQ–79 E (GSA–71)

MQ–90:

All cottons were washed on the wool scouring system; unless otherwise designated, the cottons used for this trial were from a 20-bale mix of Memphis Territory Mississippi cotton purchased commercially.

A — Unwashed control

B — Washed (lot 8, 49 °C, 40:1 water-to-fiber ratio)

C — MQ–80 cotton unwashed

D — Washed (lot 5, 49 °C, 40:1 water-to-fiber ratio)

E — Washed (lot 20, 49 °C, 20:1 water-to-fiber ratio)

F — Washed (lot 14, 49 °C, 20:1 water-to-fiber ratio)

H — Washed (lot 12, 49 °C, 20:1 water-to-fiber ratio)

I — MQ–79 C (SJ–5)

MQ–91:

Unless otherwise designated, all treatments were done on the wool scouring system using MQ–80 cotton.

A — Unwashed MQ–80 control

B — Washed (lots 7 and 8, 60 °C, 20:1 water-to-fiber ratio)

C — Washed (lots 4 and 5, 60 °C, 40:1 water-to-fiber ratio)

E — Washed (lot 1, first lot through system, extremely high water-to-fiber ratio)

F — Washed on the batch kier system (lot 1, 60 °C; lot 2, 49 °C)

MQ–95:

Unless otherwise designated, all treatments were done on the batch kier system using MQ–80 cotton.

A — Unwashed MQ–80 control

B — Unwashed MQ–80 control

C — Washed (lot 2, bale 3, 32 °C)

D — Washed (lot 2, bale 2, 32 °C)

E — Washed (lot 3, bale 3, 60 °C)

F — Washed (lot 3, bale 2, 60 °C)

H — Washed (lot 2, bale 1, 32 °C)

I — Washed (lot 3, bale 1, 60 °C)

J — Scoured and bleached—medical grade cotton washed on the continuous batt system (washed December 8, 1981)

K — Washed (lot 1, bale 1, 60 °C)

L — Washed (lot 1, bales 3 and 4, 60 °C)

MQ–101:

Unless otherwise designated, all treatments were done on the continuous batt system using MQ–80 cotton.

A — Unwashed MQ–80 control

B — High temperature washed (93 °C)

C — Low temperature washed (60 °C)

D — Scoured and rinsed (93 °C)

E — Washed and bleached (93 °C)

F — Scoured and bleached (93 °C)

G — Low temperature scoured and rinsed (60 °C)

H — Low temperature washed and bleached (60 °C)

I — Batch kier system—washed only (60 °C)

J — Batch kier system—washed and bleached (57 °C)

K — Batch kier system—scoured and rinsed (60 °C)

L — Cottonmaster™ cleaned

MQ–107:

All treatments were done on the continuous batt system using MQ–80 cotton.

A — Unwashed MQ–80 control

B — Washed and bleached (24 oz. picker laps 93 °C)

B2 — Washed and bleached (24 oz. picker laps 93 °C)

MQ–111:

All treatments were done on the continuous batt system using MQ–80 cotton.

A — Unwashed MQ–80 control

B — Scoured and bleached (93 °C)

C — Washed and bleached (93 °C)

D — Washed only (93 °C)

MQ–113:

All treatments were done on the continuous batt system using a Memphis Territory Mississippi cotton selected to have high endotoxin and gram-negative bacterial content. (Both the cotton and treatment are designated as MQ–113. The cotton was from a 25-bale mix from Staple Cotton Coop. Association, Greenwood, Mississippi.)

A — Unwashed control

B — Washed only (93 °C)

B2 — Washed only (93 °C)

H — Washed and bleached (93 °C)

I — Scoured and bleached (93 °C)

MQ–113a:

All treatments were done on the continuous batt system using MQ–113 cotton. This was a small trial to evaluate modification of the continuous batt system. The cotton from this trial was not used for human exposure.

A — See MQ–113A

B — Washed only with squeeze rolls removed, wetting agent—Washaid 1173 (93 °C)

C — Washed only with squeeze rolls in place, wetting agent—Triton X–100 (93 °C)

D — Washed only with squeeze rolls in place, wetting agent—Washaid 1173 (93 °C)

E — Washed only with squeeze rolls removed, wetting agent—Washaid 1173 (93 °C)

F — Washed and bleached with squeeze rolls removed, wetting agent—Washaid 1173 (93 °C)

G — Washed and bleached with squeeze rolls in place, wetting agent —Washaid 1173 (93 °C)

MQ–135:

All treatments were done at the Southern Regional Research Center (see chapter 7) using a mix of cotton that consisted of 247 kg of MQ–113 cotton, 191 kg of a Mississippi cotton, Grade 43 (MQ–109), and 41 kg of a Mississippi cotton, Grade 41 (MQ–109) for a total of 506 kg.

A — Unwashed control

B — Aqueous acetone washed (see chapter 7)

C — Water washed (see chapter 7)

MQ–139:

All treatments were done on the continuous batt system using 12 bales of grade 53 (low middling spot) Mississippi cotton from the 1982 crop. The cotton was blended, rebaled, and sent for washing on the continuous batt system.

A — Unwashed control

B — Washed only (finish recirculated; 93 °C)

C — Washed only (finish not recirculated; 93 °C)

D — Washed and bleached (finish not recirculated; 93 °C)

CR — Clean room, exposure

Appendix 3
Description of Test Cottons

Description item	Study number									
	79–1	MQ–79			MQ–80	MQ–89	MQ–90	MQ–113	MQ–135	MQ–139
Variety	DPL–55	DPL–61	GSA–71	SJ–5	DPL–61	Commercial cotton	Commercial cotton	Commercial cotton	Commercial cotton	Commercial cotton
Growing location	Mississippi	Mississippi	Texas High Plains	California	Mississippi	Mississippi	Mississippi	Mississippi	Mississippi	Mississippi
Staple (32nds inch)	36	34–35	32–33	36	35	35	35	34	34	35
Color grade[a]	M	SLM	M LtSp	M	SLM	SLM	SLM LtSp	—	SLM Sp	SLM Sp
Leaf grade[a]	SLM	SLM	M	SLM	SLM	SLM	SLM	—	SLM Sp	SGO
Composite grade[a]	SLM$^+$(40)	SLM (41)	M LtSp (32)	SLM$^+$(40)	SLM (41)	SLM (41)	SLM LtSp (42)	LM Sp (53)	SLM Sp (43)	LM Sp (53) BG LtSp (83)
Preparation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	—	Normal	Normal
Shirley analyzer NL (%)	2.3	2.4	2.5	2.8	2.0	2.9	3.3	3.3	2.5	4.7
Colorimeter, ginned lint										
Rd	77.6	73.5	75.0	78.8	74.4	73.4	74.8	70.5	68.6	68.0
Tb	8.8	8.0	9.2	8.6	8.9	8.6	8.9	10.8	10.1	11.5
Color	SM	SLM	M	SM	M	SLM	M	M Sp	SLM Sp	M Sp
Colorimeter										
Rd	79.4	75.5	76.1	80	77.3	75.4	76.9	72.0	72.4	72.8
Tb	8.9	8.4	9.5	9.2	8.8	9.3	9.4	11.0	10.6	11.9
Color	GM	M	SM	GM	M	M	SM	M Sp	M Sp	SM Sp
2.5% span length (in)	1.16	1.12	1.03	1.13	1.10	1.11	1.11	1.11	1.07	1.14
Uniformity ratio	40	45	44	45	45	45	45	44	43	46
Pressley strength (g/tex)	24.6	24.4	23.8	30.2	24.4	24.7	24.5	22.5	22.0	23.3
Micronaire reading	3.6	5.0	3.6	3.6	4.9	4.6	4.6	4.6	4.3	3.5
Microbiological (lint)										
Endotoxin (ng/mg)	—	0.8	0.8	0.8	—	8.3	—	26.8	15.9	130
Gram negative (cfu/mg)	—	94	1600	53	—	—	—	316.2	1660.0	—
Thermophilic (cfu/mg)	—	—	—	—	—	—	—	2000.0	—	—
Total (cfu/mg)	—	—	—	—	—	—	—	12589.0	3160.0	—

[a]See Chapter 1, table 1.6, for grade manner, codes, and symbols.

Appendix 4
Papers Published Concerning the Task Force Studies

1980

1. C. H. Chewning, Jr. Evaluation of Cleaning and Washing Processes for Cotton Fiber, Part IV: Spinning and Yarn Properties. *Text. Res. J. 50*, 79–83 (1980).
2. J. J. Fisher. Evaluation of Cleaning and Washing Processes for Cotton Fiber, Part VII: Microbiological Evaluation. *Text. Res. J. 50,* 93–95 (1980).
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4. P. E. Sasser. Evaluation of Cleaning and Washing Processes of Cotton Fiber, Part I: Introduction and Cotton Properties. *Text. Res. J. 50,* 61–63 (1980).
5. A. R. Winch. Evaluation of Cleaning and Washing Processes for Cotton Fiber, Part II: Washing Processes and Equipment. *Text. Res. J. 50,* 64–73 (1980).

1981

6. B. Boehlecke. Pulmonary Function Response to Standard and Washed Cotton. Masters Degree Thesis, University of North Carolina, Chapel Hill, 1981.
7. B. Boehlecke, J. Cocke, K. Bragg, J. Hancock, E. Petsonk, R. Castellan, and J. Merchant. Pulmonary Function Response to Standard and Washed Cotton Dust. (Abstract) *Am. Rev. Respir. Dis. 123*, 152 (1981) (Study Number: MQ79–1).
8. B. Boehlecke, J. Cocke, K. Bragg, J. Hancock, E. Petsonk, R. Piccirillo, and J. Merchant. Pulmonary Function Response to Dust From Standard and Closed Boll Harvested Cotton. *Chest 79* (4, suppl.), 77S–81S (1981).
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12. S. A. Olenchock, J. C. Mull, B. A. Boehlecke, and V. C. Major. Cotton Dust and Complement *in vitro*. *Chest 79*, (4, suppl.), 53S–55S (1981).
13. H. H. Perkins, Jr. Effects of Washing on Fiber Properties, Dust Generation and Processing Quality of Cotton. *Tex. Res. J. 51,* 123–127 (1981).

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14. J. B. Cocke. A Controlled Environmental Facility for Exposing Human Subjects to Cotton Dust. Presented at Winter Meeting of Am. Soc. Ag. Eng., 1982, paper No. 82–3576.
15. L. L. Muller, B. Piccolo, R. J. Berni, and J. B. Cocke. Chemical Characterization of Washed Cotton. *Proc. Sixth Cotton Dust Res. Conf.*, P. J. Wakelyn (ed.), National Cotton Council, Memphis, TN, 1982, pp. 47–51.
16. H. H. Perkins, Jr. The Effects of Washing Conditions on Dust Levels and Processing Quality of Cotton. Presented at Winter Meeting, Am. Soc. Ag. Eng., 1982, Paper No. 82–3578.
17. H. H. Perkins, Jr., and J. B. Cocke. Washing Methods for Cotton: Effect on Fiber, Processing Qualities and Dust Generation. *Cotton Dust: Controlling an Occupational Health Hazard,* J. G. Montalvo, Jr. (ed.), Am. Chem. Soc. Symposium Series *189*, 37–51 (1982).

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18. R. J. Berni, J. C. Montalvo, H. Perkins, and S. Buco. Differentiation of Washed and Unwashed Cotton. *Proc. Seventh Cotton Dust Res. Conf.*, P. J. Wakelyn and R. R. Jacobs (eds.), National Cotton Council, Memphis, TN, 1983, pp. 126–129.
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25. B. Boehlecke, S. Olenchock, J. Mull, J. Cocke, C. Bragg, and J. Merchant. Relationship Between Changes in FEV_1 and Endotoxin Concentration During Experimental Cotton Dust Exposures. (Abstract) *Occupational Lung Disease*, J. B. L. Gee, W. K. C. Morgan, and S. M. Brooks (eds.), Raven Press, New York, 1984, pp. 204–205.
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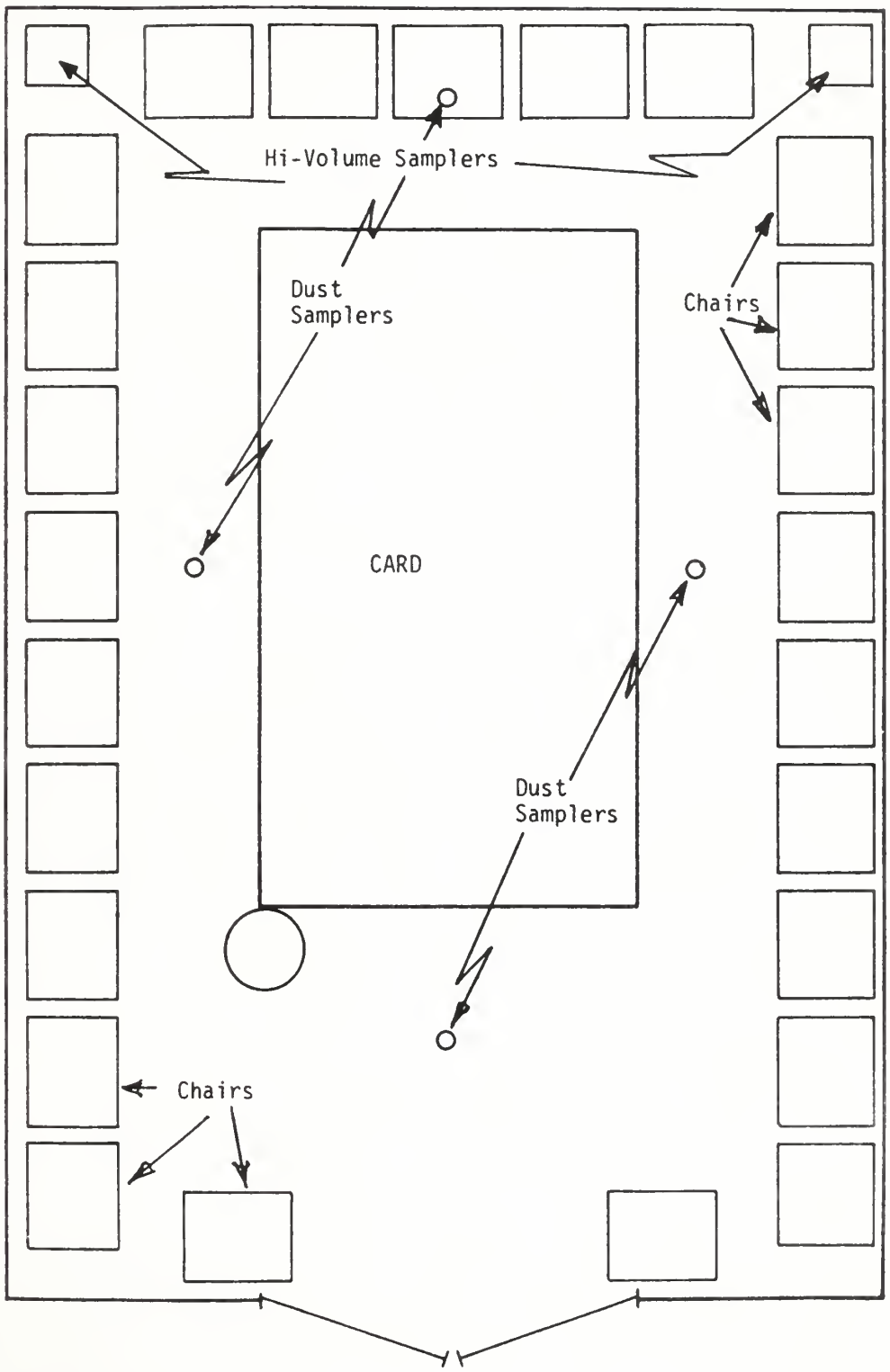
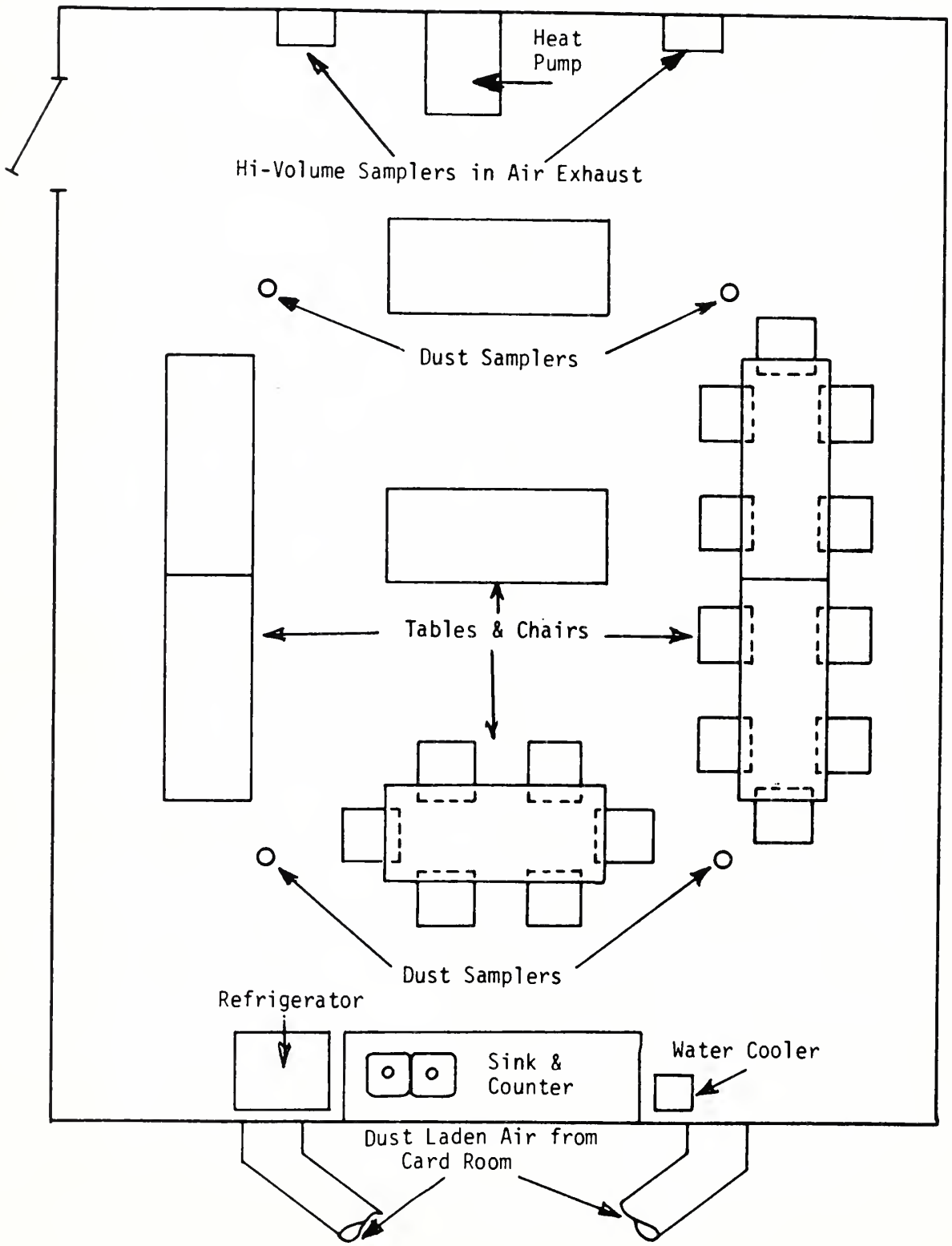
Appendix 5
Diagrams of Exposure Chambers

EXPERIMENTAL CARD ROOM



REMOTE EXPOSURE ROOM



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